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Toxic and Antagonistic Effects of Salts on Saccharomyces ellipsoideus.

Dissertation.

Presented in partial fulfilment of the requirements for the Degree of Doctor of Philosophy in the Graduate School of Ohio State University.

By

Swarna Kumar Mitra.

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Toxic and Antagonistic Effects of Salts on Saccharomyces Ellipsoideus.

Introduction.

In a former publication[126] the writer discussed the toxic and antagonistic effects of single and binary combinations of potassium chloride, magnesium chloride, calcium chloride and sodium chloride on yeast, Saccharomyces ellipsoideus. The purpose of the present paper* is to record further experiments with the same organism. The results will be discussed as follows :

First. The results of previous work on yeast by other investigators.

Second, The methods used in the present experiments with yeast.

Third. The toxic and antagonistic effects of single salts and and their binany combinations as previously reported.

Fourth. The results of ternary and quaternary combinations of four salts to show the degree of antagonism that exists in different combinations.

Fifth. Attempts to work out a physiologically balanced nutrient solution for yeast comparable with dilute grape juice.

Sixth. Degeneration of yeast cells.

Seventh. The effects of some organic substances on yeast.

Eighth. The relative osmotic pressure of salts used either singly or in combinations.

It has long been known among animal physiologists that salt solutions containing only one of the metallic chlorides, usually present in the normal media of living tissues, are highly injurious

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* These experiments were performed under the general supervision of Prof. Edgar N. Transeau in the Plant Physiology Laboratory of the Ohio State University.

to the activity of such tissues. The addition of a second chloride to such solutions diminishes the poisonous effects to a large extent. This phenomenon is usually known as antagonism. In order to have normal and long continued activity of animal tissues at least three such chlorides are essential. This has been definitely proved on the skeletal and heart muscles of vertebrates by Ringer, Howell, Green, Locke, Overton, Lingle and others.

The same phenomenon has been observed by Loew and Osterhaut working with plants and by Lipman with soil bacteria. The writer has shown in his previous publication that the different salts in single or binary combinations affect the yeast plant in a similar manner.

The composition of yeast, as analysed by Mitscherlich,[25] indicates that the inorganic constituents are chiefly phosphorus (H_3PO_4), potassium, magnesium ($Mg_3(PO_4)_2$), and a small quantity of calcium ($Ca_3(PO_4)2$).

Grape juice, which is a natural medium for the growth of yeast, constains potassium, calcium, magnesium and sodium together with small amounts of sulphur and iron.* The analyses of the organism and its natural culture medium point clearly to the inorganic salts that are necessary for yeast.

It might be thought that these analyses would furnish a basis for the making of an artificial culture medium. But, it has been found by experiment[42] that a combination of the above named salts in the ratio in which they occur in yeast or in grape juice, does not give a satisfactory growth of the organism, as measured by the amount of fermentation or by the crop of yeast.

Previous work on yeast.

Among a large number of conflcting results of experiment on the effects of various inorganic nutrient salts on yeast the following may be cited : Mayer[1] in his work with different nutrient salts

* Blyth, A. W.—Foods : Their composition analyses. p.442.

found that potasssium and magnesium phosphates in combination supply all the inorganic nutrients required for the growth of yeast. Later investigators, however, have claimed the necessity for additional inorganic salts. In his studies on yeast Linter** found that sodium and iron are not necessary for yeast culture. Molisch,[22] on the other hand, reported that iron in some form or other is essential for yeast but not calcium. This statement has been disputed by Wehmer[28] and Leyffert.*** The former maintained that iron is not necessary, while the latter found calcium to be essential for the normal growth of yeast. Kossowicz[54] has shown that calcium, magnesium and iron compounds have a decided influence on the propagation and fermentation of yeast. He found that calcium in the form of phosphate and chloride favours the growth of yeast. while iron in the form of sulphate, and to a less extent in the form of chloride, also has a favourable effect, but not to the same extent as calcium.

Later Stern[42] in his work on the nutrition of yeast reported negative results with iron. The writer's experiment on yeast gave results that agree with those of Wehmer and Stern in regard to iron, and with Leffert and Kossowicz as to the beneficial effects of calcium salts. The writer's results are also in agreement with the statement of Leyffert, that the yeast degenerates in the absence of calcium.

Kusserow[40] states that an increase of inorganic nutrients, such as calcium phosphate, potassium phosphate and magnesium sulphate, increases the fermentative power of yeast in "bad mash-material."

Heinzelmann,[34] on the other hand, has shown that the addition of such salts to a culture medium produces little or no effect on the rate of fermentation in yeast. The work of Salomen and

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** Lintner.—Lehrbuch der Bierbaur s. 427.

*** Leyffert.—Zeit. fur das ges. Brau. s. 318, 1896.

Matthews* have carroborated the results of Heinzelmann to the effect that the addition of phosphates to malt-worts decreases the assimilation of nitrogen and phosphorus by the yeast. It seems probable that the explanation for the conflicting statement of the lattter authors lies in the fact that each one of the inorganic and organic elements is favourable to the growth of organisms up to a certain concentration, above which it acts like a toxic substance. When a nutrient substance is added to a malt-wort (a physiological solution for yeast fungi) it increases the concentration of electrolytes irrespective of the concentration of the fermentable sugars, the non-electrolytes, which makes the culture medium unfavourable for the growth of yeast. As will be shown later, this has been found to be true by the writer, when nutrient salts are added to grape juice (10.11° Beaume′) in a ratio which gives the best result with the synthetic solution.

But, if grape juice is diluted to25° Beaume′ and the nutrient salts are added to it a stimulating influence is sure to result, the data for which will be given later. The malt-wort responds in the same way ; for Euler and Lundegvist[93] found that monosodium phosphate accelerated the fermentation of beer-wort but when the percentage of phosphoric acid was increased, above a certain optimum, the yeast failed to respond.

Although there is a notion current among the students of fermentation that certain inorganic salts are necessary for normal growth of yeast, very little is known as to the relative amounts of salts required or of the effects produced on the multiplication of yeast cells by variations in the proportion of such salts in solution in a culture medium. The pioneer work in this field is that of Hayduck[4]. This investigator studied the effects of nutrient salts together with varying amounts of asparagin on the weight, the amounts of nitrogen assimilated, and the fermentative power of yeast.

*Salomen and Matthews—Jour. Soc. Chem. Ind. p. 376, 1889.

Stern[42] undertook experiments on yeast with a view to determine the amount of inorganic materials required as nutrients to produce the largest crop of yeast, the greatest assimilation of nitrogen, and the most fermentation in a fixed time. He used potassium phosphate, magnesium sulphate and calcium sulphate with asparagin in a glucose medium. He found that only .025 gram of inorganic salts is required for the best growth per 100 cc of the sugar solution. The growth of the yeast here was undoubtedly due to asparagin which is assimilated by yeast very rapidly even with a minimum concentration of inorganic salts. Laurent[15] found that asparagin is assimilated even in the absence of any carbohydrate and Loew* has found it to be a good source of carbon for both bacteria and fungi.

Results of investigations on the effects of salts of heavy metals on yeast, such as mercuric chloride, silver nitrate etc., have been reported by Bokorny,[103] but nothing on the toxic and antagonistic effects of such salts as sodium chloride, potassium chloride, calcium choride and magnesium chloride. This field, therefore, seemed inviting, especially from the botanical stand-point, and it was with the idea of studying the physiological relations existing between yeast fungi and the chorides both singly and in combination that the writer undertook the work summarised in the following pages.

Methods used in the experiments on yeast.

The methods adopted in the experiments with single salts and their combinations may be briefly stated as follows :

The particular pure culture of yeast No. 66, which the writer has used throughout the previous and present experiments, was isolated by proof W. V. Cruess of the University of California.

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* Loew, O.—Die Chemische Energie der lebenden Zellen. Munchen, s, 175,1899 ; Bied. Centbl., Bd. 29,s.142,1899.

In order to grow the yeast in a medium whose constituents are known, it is necessary to prepare a nutrient solution from chemically pure materials. As the yeast requires an adequate amount of nitrogen and phosphorus on a carbohydrate base, pure cane sugar was hydrolyzed with phosphoric acid and neutralized with dilute ammonium hydroxide. Thus, the amonium phosphate that eventually formed furnished both the nitrogen and phosphorus needed by the yeast fungi. This synthetic solution gave a suitable growth for experimental purposes. Although the rate of growth in this case was very slow as compared with grape juice, it was sufficient for microscopic counting. .

In order to prepare this synthetic solution a 50% pure cane sugar syrup was made with distilled water and one gram of phosphoric acid was added per 100 cc of the syrup. This syrup was hydrolyzed by boiling for one-half hour and was then neutralized with dilute ammonium hydroxide. As the yeast fungi grow better in a mederately acid solution, the syrup was left slightly acid (.2 to .3 %) being tested by titration with N/10 NaOH. To facilitate' the work the syrup was boiled down to 32° Beaume' and was put up in an air-tight bottle so as to free it from bacteria and moulds. From this concentrated synthetic solution, a measured quantity was drawn off and diluted with distilled water to 2.5° Beaume' for use in the cultures.

Method of determining the effects of salts on yeast.

The experiments were carried on in a series of 250 cc Erlenmyer flasks. To each flasks the weighed amount of salts was added and 100 cc of the diluted (2.5° Beaume') synthetic solution was placed in the flask by means of a 100 cc pipette.

The salts were weighed according to their respective molecular concentrations. The flasks were all carefully plugged

with cotton and sterilized. They were then inoculated with yeast from a new culture in synthethic medium. For this purpose the new culture was transferred from a mother culture in grape juice, the latter of which was renewed from time to time, as the yeast degenerates in a culture flask after a period of several weeks. The new culture after inoculation was incubated for forty-eight hours at 28° C. The yeast fungi thus became habituated to the synthetic medium and therefore grew more uniformly in the experimental flasks. At the end of this period the flasks containing the salts were inoculated with one cubic centimeter of the new culture. After inoculation these experimental flasks were put into the incubator which was kept at a temperature of 28° C during the entire period of experimentation, although sometimes it differed by a degree or two which, however, did not materially interfere with the uniformity of growth and the check flasks showed any variation it may have caused.

It has usually been customary for investigators to take the amount of alcoholic fermentation in the culture flasks as the criterion to determine the activity of the yeast organism. As the purpose of the writer here was to determine the rate of growth by the multiplication of the yeast cells, as accurately as possible, instead of fermentation of the same, any attempt primarily to have a heavy culture was avoided. The object was to grow a light culture of yeast and directly determine the rate of growth by microscopic counting irrespective of fermentation. Accordingly, as the alcoholic fermentation of the synthetic solution in culture flasks was very slow the multiplication of yeast cells was taken as the measure of activity. For this purpose a microscopic counting was made, using a Zeiss haemacitometer, such as is generally used in blood counting. One such counting was made every forty-eight hours in each experiment during a period of twelve days. In every case blank cultures were made so as to check up the uniformity of growth in all the culture flasks, and the results have

been shown in curves which represent the average of two sets of duplicate experiments.

It may be added here that in microscopic counting one million yeast cells per cubic centimeter was taken as the lowest appreciable number.

It is a common practice among the physiologists to use nutrient salts in the form of a liquid, as measured from a solution of known molecular strength, while in all my experiments I have added the salts directly to the culture flasks according to their respective molecular weights. The single salts and their different combinations were carefully weighed and were put directly into the flasks. This gave a known molecular concentration without diluting the synthetic solution.

All of these solutions were clear except the higher concentrations of calcium chloride, which gave an appreciable precipitate of calcium phosphate with the phosphorus of the synthetic solution. This, however, did not interfere with the experiment, as the precipitation disappeared with the growth of yeast and the solution finally became clear. The fact is that the yeast produces an acidity of from .5 % to 1.3 % during the period of twelve days. It is this increase of acidity in a culture flask which dissolves the precipitated calcium phosphate and ultimately not only checks the growth of yeast, but even brings about an autolysis by which the yeast cells begin to degnerate rapidly. This deleterious effect of acidity on yeast will be considered in detail later.

Results obtained with single salts and their binary combinations.

In the previous publication the writer dealt with the toxic and antagonistic effects of single salts and their binary combinations. The four single salts used were potassium, magnesium, calcium and sodium chloride. The experiments were arranged in two series, the first dealt with the toxicity of single salts and the second

with the antagonistic effects of their binary combinations as follows :

1. Toxicity of single salts.
   1. KCl
   2. $MgCl_2$
   3. $CaCl_2$
   4. NaCl
2. Antagonistic effects of binary combinations.
   1. $MgCl_2 + CaCl_2$
   2. $KCl + CaCl_2$
   3. $MgCl_2 + NaCl$
   4. $KCl + NaCl$
   5. $KCl + MgCL_2$
   6. $CaCl_2 + NaCl$

The toxic effects of single salts were found to be as follows : (1) Each one of the four single salts is more or less toxic to yeast. At a certain concentration potassium chloride is the least toxic and sodium chloride, the most. (2) The lower concentrations of each salt stimulate the growth of yeast more or less, while the higher concentrations inhibit it markedly. The optimum concentration of each salt, as measured by growth, was found to be .2 M KCl, .1 M $MgCl_2$, .01 M $CaCl_2$, and .001 M NaCl. The concentrations at which the growth is entirely inhibited were 2.2M KCl, 1.2 M $MgCl_2$, .7 M $CaCl_2$, and .2 M NaCl.

The results here are rather different from those found by other investigators for bacteria, other plants and animals. Concerning the experiments conducted with other plants, the adaptability of yeast to salts was found to be remarkable, especially at higher concentrations which hardly allow any other plant to grow in it. Besides, it was found that with the increase of toxicity of salts the yeast cells decrease in size, until a point is reached where the budding of cells is entirely inhibited.

The antagonistic effects of the binary combinations, i. e., where the different salts were used in pairs, were found to be arranged in order from the highest to the lowest as follows : (1) $MgCl_2 + CaCl_2$, $KCl + CaCl_2$, $MgCl_2 + NaCl$, $KCl + NaCl$, $KCl + MgCl_2$, and $CaCl_2 + NaCl$. Of these six combinations

antagonism was quite pronounced in the first three combinations, while in the last three, it was only slight. (2) The writer's data do not indicate that there is a valency law effect as claimed by Loeb. Di-valent ions may antagonise both di-valent and mono-valent ions, and mono-valent ions may antagonise di-valent ions and to a slight extent mono-valent ones. It may safely be stated that whenever a minimum concentration of salt is added to another, there is always more or less antagonism.

In general, the results with binary combinations on yeast is similar to those recorded for bacteria, other plants and animals, although the degree of antagonism varies somewhat with different organisms at the same concentration of salts. This rather justifies the conclusion of Hawkins[113] that no broadly general hypothesis will explain the various antagonistic relations that have been observed by different investigators up-to-date on different organisms and that the explanation in each case will have to be worked out separately.

Experiments with ternary and quaternary combinations of salts.

In order to determine the effects of ternary and quaternary combinations of salts on yeast the following series of experiments were arranged :

A. Ternary combinations.
   1. $KCl + CaCl + NaCl$.
   2. $MgCl_2 + CaCl_2 + KCl$
   3. $KCl + MgCl_2 + NaCl$.
   4. $MgCl_2 + CaCl_2 + NaCl$.

D. Quaternary combinations.
   1. $KCl + CaCl_2 + NaCl + MgCl_2$.

A. Experiments with ternary combinations of salts.

In previous experiments the writer found that when only one salt is used in culture media, the yeast becomes adjusted to it at low concentrations and shows no toxic effect until a certain concentration is reached. When a second salt is added to the first, not only is the toxicity of the single salt reduced by the presence of the other, but the combined effect of the two salts is less than the sum of their individual effects, although the total molecular concentration of two salts and the osmotic pressure of the medium are simultaneously raised higher than those of single salt media. Similarly, the yeast becomes adjusted, as with single salts to assimilate the two salts until the medium becomes toxic at a certain concentration. If such a binary combination that gives the best result is taken as a standard and a third salt is added to it, the molecular concentration of the three salts and likewise the osmotic concentration of the medium is increased to such a high degree that the total effect of three salts in combination instead of accelerating the growth of the organism becomes additive. In order to avoid this difficulty the writer has chosen the lower concentration of salts in the same ratio in which the combinations were found to give the greatest growth of yeast cells in each series and employed these in the ternary experiments at a constant ratio against the third. This was primarily done to show the antagonism of the binary combinations against the third salt and vice versa.

Series 1. Antagonism of $KCl + CaCl_2 + NaCl$.

In this series the following three combinations of three salts each are possible :

A. $KCl + CaCl_2 + NaCl$
B. $KCl + NaCl + CaCl_2$
C. $CaCl_2 + NaCl + KCl$.

All of these combinations were tried. For each combination a number of 25 cc Erlenmyer flasks were used in duplicates including blank cultures. For example, for the combination A the first pair of flasks had no salt and was used as a check. The second pair had the binary combination of .016 M KCl and .01 M $CaCl_2$ which was also used as check, as it were, against the third salt added. The third pair contained .016 M KCl + .01 M $CaCl_2$ + .001 M NaCl and so on with the rest of the mixtures. All the flasks, except the blanks, contained the constant proportion of .016 M KCl and .01 M $CaCl_2$, while NaCl was added from .001 M to .8 M, as is shown in curves of Fig. 1.

The other two combinations were tried in the same way. Among these three combinations antagonism was noticed in all cases, but was most pronounced in A and least in C. As all the combinations are of a similar type, having the same three salts in each case, B and C have been omitted from the discussion of results.

The curves have been plotted from the results of every forty-eight hour's growth, as has been done in previous experiments. For example, the ordinate at O represents the binary combination of .016 M KCl + .01 M $CaCl_2$, at .001, the ternary combination of .016 M KCl + .01 M $CaCl_2$ + .001 M NaCl, while the rest represent the addition of NaCl from .01 M to .8 M to the constant ratio of .016 M KCl and .01 M $CaCl_2$. This has been arranged in such a way as to determine the antagonistic effects of KCl and $CaCl_2$ vs. NaCl and vice versa.

From the curves in Fig. 1 it is evident that there is a distinct antagonism of KCl + $CaCl_2$ + NaCl. For example, the binary combination of .016 M KCl + .01 M $CaCl_2$ allowed a growth up to 30 millions, while the same combination with .001 M NaCl stimulated the growth up to 45 millions. Similarly, .2 M NaCl alone inhibited the growth of yeast entirely, but when .016 M KCl and .01 M $CaCl_2$ were added the growth was increased to 25

Fig 1

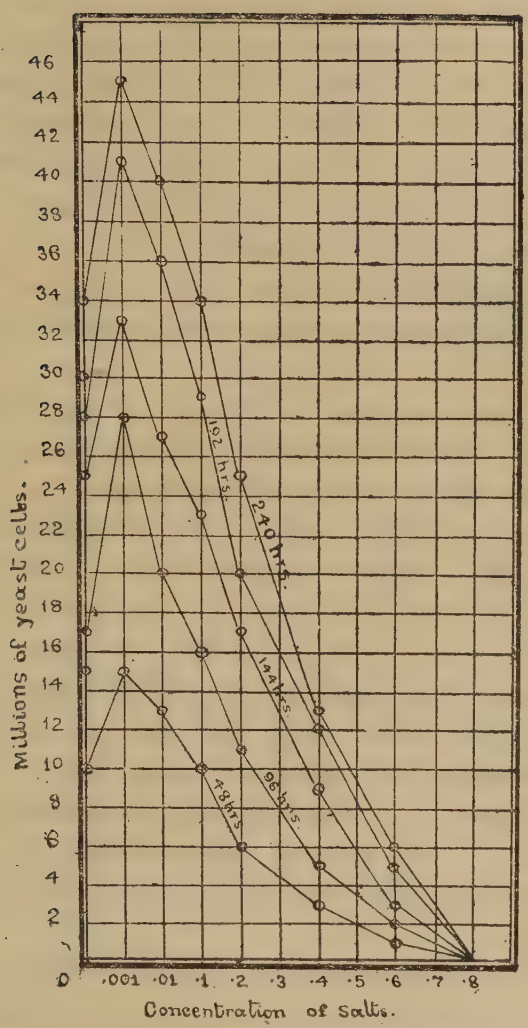

Fig. 1. Curves of yeast growth showing antagonism of $KCl + CaCl_2 + NaCl$. The ordinates represent the number of yeast cells in millions and the abscissae, the concentration of salts in combination. The ordinate at O represents the number of yeast cells of the binary combination of KCl and $CaCl_2$ in the ratio of 1.6:1, while the rest represent the addition of NaCl from .001 M to .8 M.

millions. This shows that the antagonism on the side of NaCl was much pronounced until at the extreme concentration of .8 M NaCl, where the yeast ceased to grow entirely. Here the inhibitory effect of .2 M NaCl alone has been raised to the level of .8 M NaCl by the addition of small amounts of KCl and $CaCl_2$. In this series the highest growth was obtained at the concentration of .016 M KCl + .01 M $CaCl_2$ + .001 M NaCl in the ratio of 1. 6: 1: .1 respectively.

For comparison let us now consider the results obtained by other investigators in similar experiments on plants and animals :

(a) Plants —Osterhaut [65, 70] found a distinct antagonism among these three salts in certain combinations. He showed that marine algae die sooner in NaCl solution (isotonic with sea water) than in distilled water. The poisonous effect of 1000 cc $\frac{3}{8}$ M NaCl largely disappears if 10 cc $\frac{3}{8}$ M $CaCl_2$ is added. In this combination the plants live as long as in distilled water. But when 22 cc $\frac{3}{8}$ M KCl is added the three salt solution enables the plants to live a considerably longer period than in distilled water. This was also true in the case of wheat, equisetum and cuttings of higher plants. From this he seems to derive his conclusion that the combination of NaCl, $CaCl_2$ and KCl in the same ratio in which they occur in sea water is quite beneficial to plants as has been found by Loeb for animals.

In their experiments on the ratio of phosphate, nitrate and potassium on the absorption and growth of wheat seedlings Schreiner and Skinner[59] have found that the three metallic ions of Na, Ca and K in Combination with their respective anions at certain molecular concentration give the best result.

(b) Animals.—Loeb[13] with a marine fish (Fundulus) observed a distinct antagonism in a certain combination of these three salts. He found that the fishes die within 24 hours in

a $\frac{5}{8}$ M NaCl+$\frac{5}{8}$ M $CaCl_2$, but when 2cc $\frac{3}{8}$ M KCl is added, they live more than ten days.

He also observed a similar phenomenon with the eggs of same fishes[60] and Arbacia.[68] Besides, with a jelly fish (Gonionemus) Loeb[43] showed that the same concentration of three salts in combination produced rhythmical contraction in the swimming bells of the organisms lasting for an hour or more which was entirely inhibited by NaCl alone at the same concentration. Moreover, the same investigator[44] with the eggs of sea ucrhins found a similar behaviour for these three salts.

Anne Moore[45] has corroborated the work of Loeb on young trout. In this case the combination of 100 cc $\frac{1}{8}$ n NaCl + 2 cc $\frac{2}{3}$ n $CaCl_2$ + 2 cc $\frac{1}{8}$ n KCl gave the best result. Besides, Cushing[50] has shown that a solution containing .7% NaCl,. 03% $CaCl_2$ and .03% KCl is a balanced one for the contraction of muscular tissues of frogs.

Lillie[52] with Arenicola larvae found that a salt solution containing 20 cc $\frac{4}{8}$ n NaCl + 3 cc $\frac{4}{8}$ n KCl + 5 cc $\frac{4}{8}$ n $CaCl_2$ enables the muscular movement of the organism which is not so pronounced in a combination of two salts and is entirely inhibited by one alone.

Ostwald[58] on fresh water animals (Gammarus) found that a combination of NaCl, KCl and $CaCl_2$ at a certain concentration approaches sea water in which the organism can live normally.

A negative result has been reported by Loeb on Hydromedusa gonionemus[45] in which the combination of three salts prevented the marginal rhythmical contraction which was produced by NaCl alone. This was contrary to the central beating of the same organism, which was inhibited by NaCl but was produced by a mixture of the three salts very markedly.

From the above discussion of plants and animals it is evident that there is a distinct antagonism of $KCl + CaCl + NaCl$ on both sides of the curves, in which the antagonism of higher concentration of NaCl against a comparatively lower concentration of KCl and $CaCl_2$ is most pronounced. Besides, coosidering the work of Loeb and Osterhaut it may safely be stated that the toxic effect of Na ions is antagonised by the addition of K and Ca ions at a certain molecular concentration in which the yeast shows a vary concordant result.

It may be added here that sodium is the most toxic to yeast and later experiments will show that it is least needed by the organism as a nutrient salt, but the presence of Na ions in such a synthetic solution removes the toxicity of other electrolytic ions as K and Ca and non-electrolytes, such as sugar, the latter not giving sufficient growth of yeast when used alone in distilled water and thus behaving toxically. Loeb[106] found a similar effect when the toxicity of sugar solution was removed by the addition of salts (NaCl) on the marine fish (Fundulus). The physical effects of the combination of KCl, $CaCl_2$ and NaCl on yeast agree, in general, with those of other plants and animals.

SERIES 2 ANTAGONISM OF $MgCl_2 + CaCl_2 + KCl$.

The experiments in this series were carried on in the same way as the previous ones. In this also three possible combinations of three salts each were arrranged and tried as follows :

A. $MgCl_2 + CaCl_2 + KCl$

B. $CaCl_2 + KCl + MgCl_2$

C. $KCl + MgCl_2 + CaCl_2$

All these combinations showed antagonism more or less of which the combination A gave the best result as was comparable, so far as the multiplication of yeast cells is concerned, to the results obtained in the previous series. As all the three combinations are similar, the combination A has been taken for discussion,

while B and C have been omitted, as has been done before with series 1.

The first two salts in the case of A have been taken at the constant ratio of .016 M KCl and .01 M $CaCl_2$, while the third salt (KCl) has been added from .001 M to .8 M. The results are shown in curves of Fig. 2.

From the curves it is evident that there is a certain degree of antagonism in this combination of $MgCl_2 + CaCl_2 + KCl$. For example, .022 M $MgCl_2$ + .01 M $CaCl_2$ produced a growth of 32 millions, but with the addition of .01 M KCl the growth was increased to 42 millions. Similarly, there was also an antagonism on the side of KCl. Because, .01 M KCl alone allowed a growth of 24 millions, while in combination with .022 M $MgCl_2$ + .01 M $CaCl_2$ the growth had been raised up to 35 millions. This antagonism on the side of KCl was not as marked at higher concentrations as was found with NaCl in the previous series. In this series the yeast continued to slow down in growth up to the concentration of 1.6 M KCl, beyond which no appreciable amount of growth was noticed. Besides, the combinations of .022 M $MgCl_2$ + .01 M $CaCl_2$ + .01 M KCl gave the best result in the ratio of 2.2 ; 1 : 1 respectively.

In comparison to the results obtained in this experiment a number of cases dealing with plants and animals may be cited below :

(a) Plants. — In their work on spyrogyra Loew and Aso [73] reported a negative result in which the combination of .01 per mille K_2SO_4 + .04 per mille $Ca(NO_3)_2$ + .02 per mille $MgSO_4$ did not act as a better medium than two salts in combination, thus showing a lack of antagonism among the three metallic ions.

True and Bartlett [125] with the seedlings of Lupinus albus found that the plants absorb more salts in a combination of $KNO_3 + Ca(NO_3)_2 + Mg(NO_3)_2$ than from equally concentrated

Fig. 2.

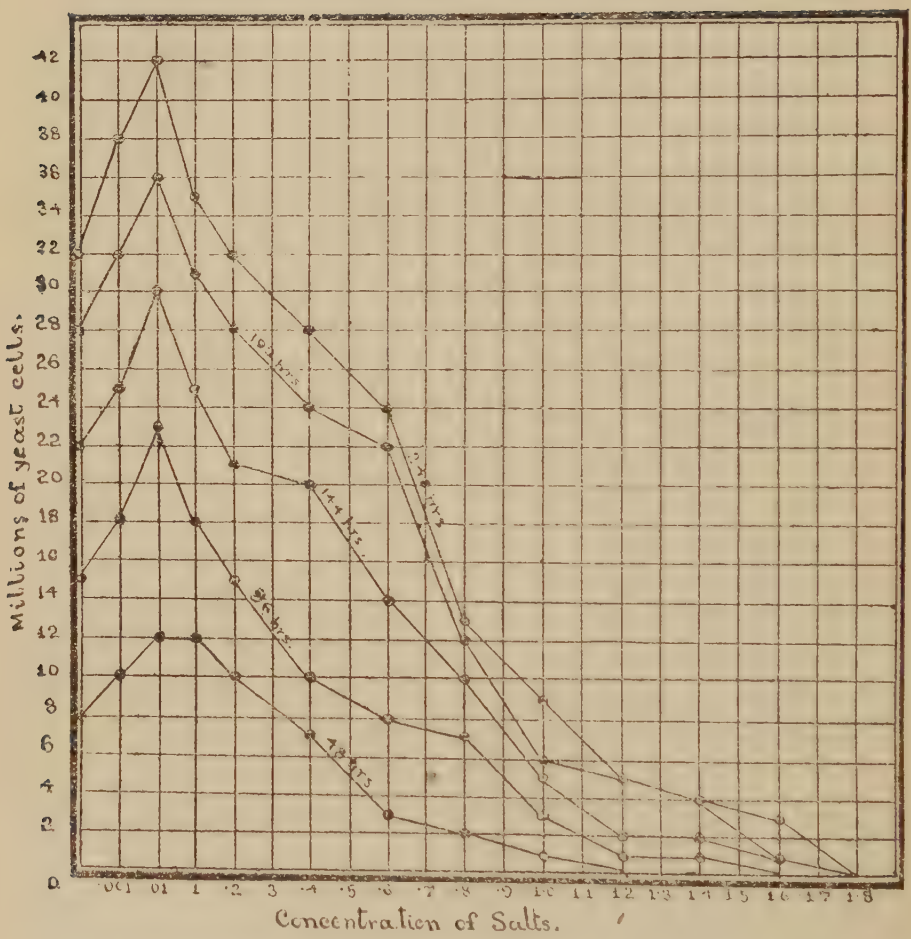

Fig. 2. Curves of yeast growth showing antagonism of $MgCl_2 + CaCl_2 + KCl$. The ordinates represent the number of yeast cells in millions and the abscissae, the concentration of salts in combination. The ordinate at O represents the number of yeast cells of the binary combination of $MgCl_2$ and $CaCl_2$ in the ratio of 2.2: 1, while the rest represent the addition of KCl from .001 M to .1 M.

solution containing one or two nitrates. This means a certain degree of antagonism of the three metallic ions in combination.

(b) Animals. — With the unfertilised eggs of sea urchins (Arbacia) Loeb [44] has shown that the segmentation of eggs does not occur in a mixture containing 76 cc 10/8 n $MgCl_2$ + 2 cc 10/8 n $CaCl_2$ + 2 cc 5/8 n KCl, thus showing a lack of antagonism in a combination of these three salts.

The results of these series of experiments show that although there is no direct evidence of antagonism among these three chlorides in other plants or animals, there is a certain antagonistic effect shown by yeast.

Series 3. Antagonism of $KCl + MgCl_2 + NaCl$

The cultures in this series have been treated in the same general way as before. The three possible combinations of three salts each are as follows :

A. $KCl + MgCl_2 + NaCl$
B. $KCl + NaCl + MgCl_2$
C. $NaCl + MgCl_2 + KCl$

All of these three-salt combinations were tried and in every case there was antagonism more or less. It was most pronounced in A and least in C. As the salts in each case are similar the combination A has been taken for discussion, while B and C have been omitted.

In combining the salts of A the first two have been taken at the constant ratio of .01 M KCl + .015 M $MgCl_2$ against the third (NaCl) which has been taken from .001 M to .8 M, as is shown in curves of Fig. 3.

From the curves it is evident that there is a decided antagonism in this series. For instance, the combination of .01 M KCl + .015 M $MgCl_2$ allowed a growth up to 22 milions, while the addition of .001 M NaCl stimulated the growth up to 33 millions. Similarly, on the side of NaCl the antagonism was very markedly

high, as has been found in series 1. For, .2 M NaCl hardly allowed any growth when used alone, but in combination with .01 M KCl + .015 $MgCl_2$ the growth was increased to 20 millions. The rest of the combinations with higher concentrations of NaCl showed a similar result until at the point of .8 M NaCl, the yeast.

Fig. 3

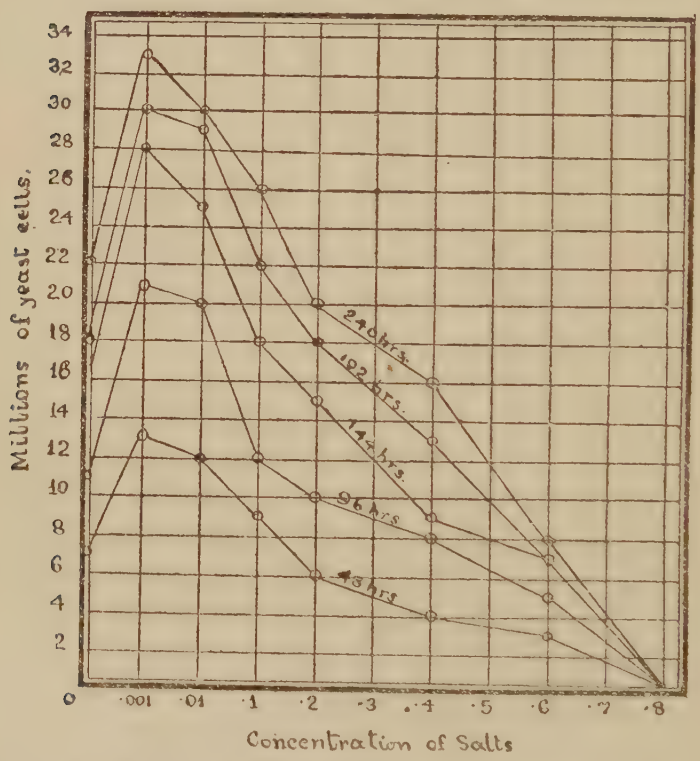

Fig. 3. Curves of yeast growth showing antagonism of KCl + $MgCl_2$ + NaCl. The ordinates represent the number of yeast cells in millions and the abscissae, the concentration of salts in combination. The ordinate at O represents the number of yeast cells of the binary combination of KCl and $MgCl_2$ in the ratio of 1: 1. 5, while the rest represent the addition of NaCl from .001 M to .8 M.

ceased to grow entirely. In this series the highest growth was obtained in the combination in which the molecular concentration of three salts have been used in the ratio of 1: 1 .5: .1.

For comparison a number of cases dealing with bacteria, plants and animals may be cited below :

(a) Bacteria. — Lipman [87] found that the use of .35 M solution of 100 cc NaCl + 10 cc $MgCl_2$ + 25 cc KCl gives a more satisfactory result in ammonification by the soil bacteria (B. subtilis) than with one or two salts in combination.

(b) Plants. — Osterhaut [65, 79] in his work on other plants found that the combination of 1000 cc $\frac{3}{8}$ M NaCl + 78 cc $\frac{3}{8}$ M $MgCl_2$ + 10 cc $\frac{3}{8}$ M KCl is toxic to most of the algae and also to wheat seedlings and cuttings of higher plants. But in the case of wheat no additive effect was shown. The same investigator [105] with the algae, Enteromorpha hopkirkii, found an antagonism to a certain extent in the same three-salt mixture.

Hansteen [90] experimented on the relation of plants to the salts of the soil on wheat seedlings. He showed that the mixture of $MgCl_2$ + NaCl + KCl at a certain molecular concentration makes the roots to shrivel and they die after a time. Neither temperature, osmotic pressure, nor the anions present influence the toxicity of salts.

(c) Animals. — Loeb [43] with a marine fish (Fundulus) observed a distinct antagonism of the three salts in combination, which allowed the organism to live for a few hours, while the single salts or their binary combinations killed them in a short time. The same investigator [43] with a jelly fish (Gonionemus) found a rather contradictory result. But he [69] also found a positive result in another jelly fish (Polyorchis) , when the same concentration of salts was used.

Lillie[52] with Arenicola larvae found a certain degree of antagonism with this three-salt mixture. He showed that a combination of NaCl, KCl and $MgCl_2$ at certain molecular

concentration is more favourable for the muscular and ciliary movement of the organism than one or two salts at the same concentration.

Ostwald[53] on fresh water animals (Gammarus) found a lack of antagonism in relation to the duration of life of that organism at a certain molecular combination of these three salts. However, it did not show any more inhibitory effect than two salts in combination.

From the above discussion it is evident that there are positive and negative results in both plants and animals. The yeast shows a certain degree of antagonism.

Series 4. Antagonism of $MgCl_2 + CaCl_2 + NaCl$.

The plan of this series was the same as has been followed with the others. In the case of binary combination of $MgCl_2 + CaCl_2$ a marked antagonism was found between these two salts, but the addition of NaCl gave a rather negative result. However, a high degree of antagonism was found on the side of NaCl, as has been shown before in series 1 and 3.

In this series also the three possible combinations of three salts each were tried as follows :—

A. $MgCl_2 + CaCl_2 + NaCl$
B. $MgCl_2 + NaCl + CaCl_2$
C. $CaCl_2 + NaCl + MgCl_2$

In all these combinations the general growth was very low in comparison to other series. As the salts are similar in each case. only the combination A has been taken for discussion, while B and C have been omitted. As with the previous series, the first two salts have been taken at a constant ratio of .022 M $MgCl_2$ + .01 M $CaCl_2$, while NaCl has been added from .001 M to .8M, as is shown in curves of Fig. 4.

The results indicate that the combination of .022 M $MgCl_2$ and .01 M $CaCl_2$ allowed a growth of 32 millions, while the

addition of .001 M NaCl did not increase the growth at all, But, however, this was just the opposite on the side of NaCl. Experiments with single salts have shown that .2 M NaCl hardly allowed any growth of yeast, while the addition of .022 M $MgCl_2$ + .01 M $CaCl_2$ raised the growth up to 19 millions. In this case the growth was entirely checked at .8 M NaCl with the

Fig 4.

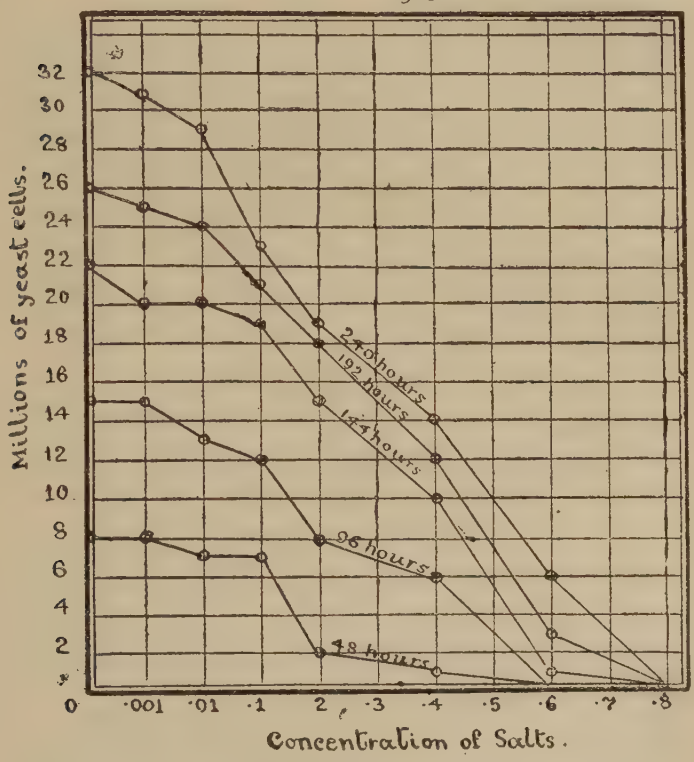

Fig. 4. Curves of of yeast growth showing antagonism of $MgCl_2 + CaCl_2 + NaCl$. The ordinates represent the number of yeast cells in millions and the abscissae, the concentration of salts in combination. The ordinates at O represent the number of yeast cells of the binary combination of $MgCl_2 + CaCl_2$ in the ratio of 2.2:1, while the rest represent the addition of NaCl from .001 M to .8M.

binary cambination of $MgCl_2$ and $CaCl_2$, which altogether produced the same inhibitory effect as with .2 M NaCl alone.

For comparision with other investigators the following cases may be cited ;

(a) Bacteria.—Lipman[87] with soil bacteria found that a combination of .35 M solution of 100 cc NaCl + 10 cc $MgCl_2$ + 5 cc $CaCl_2$ gives a better result in ammonification than NaCl and $MgCl_2$ together.

(b) Plants.—Osterhaut[65,70] has shown that a combination of 1000 cc $\frac{3}{8}$ NaCl + 78 cc $\frac{3}{8}$ M $MgCl_2$ + 10 cc $\frac{3}{8}$ M $CaCl_2$ is better suited to the growth of marine algae than the combinaiion of NaCl, $MgCl_2$ and KCl at the same concentration. But in the case of wheat a negative result was obtained by the same salts at the same molecular concentration. In some cases of algae the combination, however, approached sea water in which the plants were found to live normally.

(c) Animals—Loeb [43] with Fundulus found that in a combination of 96 cc $\frac{5}{8}$ n NaCl + 2 cc $\frac{10}{8}$ n $MgCl_2$ + 2 cc $\frac{5}{8}$ n $CaCl_2$ the animals live longer than in one or two salts. Similarly, he [43] found with a jelly fish (Gonionemus) that the above mentioned salts in the same proportions caused an appreciable muscular contraction, whose period was normal lasting for an hour or more, while one or two salts stopped it entirely. The same investigator[69] found a negative result on another jelly fish (Polyorchis) in a mixture of the same salts.

Lillie [52] has conculsively shown with Arenicola larvae that a combination of 80 cc $\frac{5}{8}$ n NaCl + 15 cc 10/8 n $MgCl_2$ + 5cc 10/8 n $CaCl_2$ gives the best result among all the ternary combinations in setting up a ciliary movement of the organism.

Ostwald [58] with fresh water Gammarus found a negative result, in a combination of these three salts. In this case the addition of $MgCl_2$ to NaCl and $CaCl_2$ gave an additive effect, thus showing a lack of antagonism.

In all cases cited above both positive and negative results were found in plants and animals. The yeast, however, shows here an inhibitory effect in agreement to the results obtained by Osterhaut on wheat, Loeb on Polyorchis and Ostwald on Gammarus, although a pronounced antagonism was shown on the side of NaCl, as has been found in series 1 and 3.

From above experimental data it is evident that in all cases of ternary combinations the mixture of KCl, $CaCl_2$ and NaCl in the ratio of 1.6: 1: .1 respectively, as is shown in series 1, allowed the highest growth with a maximum degree of antagonism. In all cases, where NaCl was used in different molecular concentrations from .001 M to .1 M, a marked antagonism was shown.

In their work with inorganic salts investigators have always tried to replace one salt by another which has been found to be partially successful in a few cases. Bokorny has shown that in plant life the place of one element cannot be taken by another. The role played by each metallic element, especially those that have been used with yeast is probably a definite one.

In regard to yeast the metallic ions, such as K, Ca, Mg and Na are probably not all needed by the organism, but are required to antagonise the toxicity of one salt by the other. The above four series of experiments show that K and Ca ions are most needed by the yeast plant. When either of these two elements is lacking the growth falls, as is evident from data given. Neither sodium nor magnesium can take the place of potassium or calcium. When potassium is lacking the yeast crop becomes decidedly low, while for want of calcium the yeast decreases in size, appears unhealthy and degenerates rapidly. The part played by magnesium ions will be shown in the following experiment.

B. Experiments with quaternary combinations of salts.

In the experiments with quaternary salts the writer will try to show the nature of antagonism that exists among the four chlorides in combination, as has been shown in the case of ternary combinations.

From the four chlorides four combinations of four salts each are possible, namely,

A. $KCl + CaCl_2 + NaCl + MgCl_2$.
B. $KCl + MgCl_2 + NaCl + CaCl_2$.
C. $MgCl_2 + NaCl + CaCl_2 + KCl$.
D. $MgCl_2 + CaCl_2 + KCl + NaCl$.

All of these combinations were tried and in every case an antagonism was found to be more or less evident. The rate of growth was rather uniform in every case although the concentration of salts varied. However, the antagonism was shown most in A. As the combinations contain the same four salts in each case, only the combination A has been taken for discussion of results, while B, C and D have been omitted, as has been previously done in the case of ternary combinations.

Series 5. Antagonism of $KCl + CaCl_2 + NaCl + MgCl_2$.

In the selection of salts for the quaternary combination of $KCl + CaCl_2 + NaCl + MgCl_2$, the ternary combination of series 1 that gave the highest growth among all the ternary combinations was chosen for experiment in this case and the fourth salt was added in various molecular concentrations. Accordingly, the mixture of .016 M KCl + .01 M $CaCl_2$ + .001 M $NaCl$ was taken at a constant ratio and $MgCl_2$ was added from .001 M to .8 M. The results are shown in the curves of Fig. 5.

From the curves of Fig. 5 it is evident that there is a distinct antagonism among these four salts in combination. For example,

Fig 5

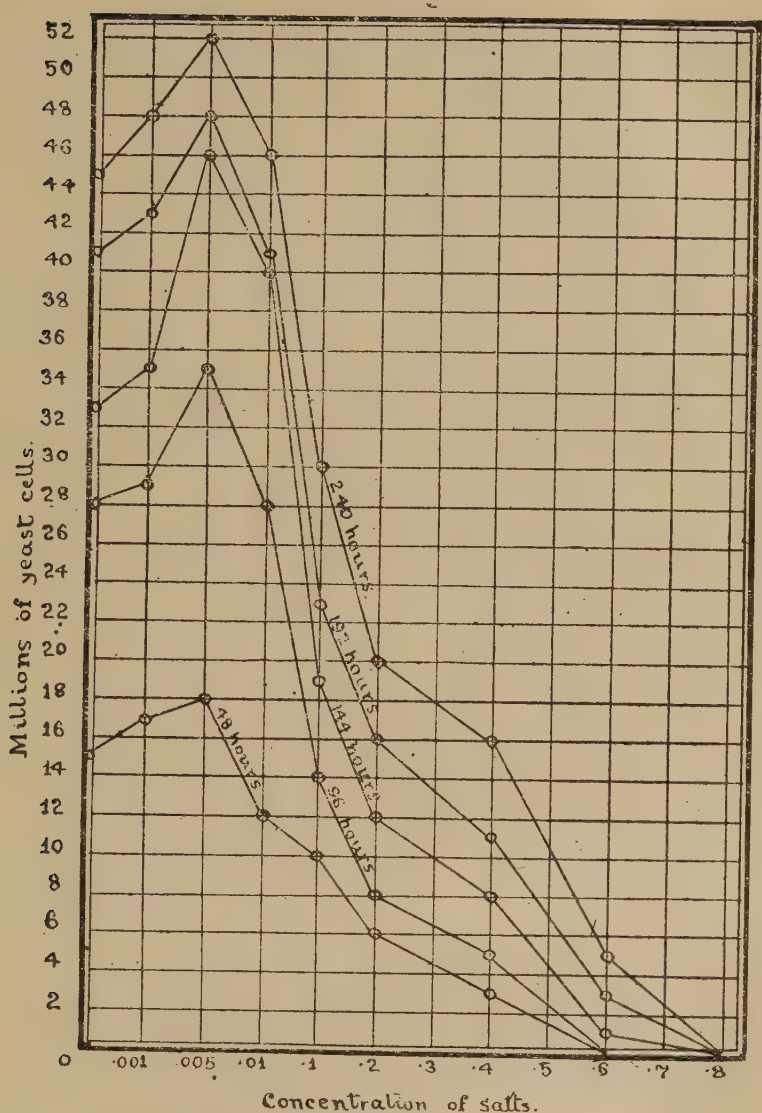

Figure 5. Curves of yeast growth showing antagonism of $KCl + CaCl_2 + NaCl + MgCl_2$. The ordinates represent the number of yeast cells in millions and the abscissae, the concentration of salts in combination. The ordinate at O represents the number of yeast cells of the ternary combination of $KCl + CaCl_2 + NaCl$ in the ratio of 1·6 : 1 : ·1, while the rest represent the addition of $MgCl_2$ from ·001 M to ·8M.

the ternary combination of .016 M KCl + .01 M $CaCl_2$ + .001 M NaCl allowed a growth of 45 millions, while the addition of .005 M $MgCl_2$ stimulated it up to 52 millions. Besides, on the side of $MgCl_2$ the antagonism was very high indeed. For instance, .01 M $MgCl_2$ alone allowed a growth up to 16 millions, while in combination with three other salts it was raised up to 46 millions. However, the higher concentrations of $MgCl_2$ even at .2 M did not show any antagonism and instead acted toxically. Such antagonism was noticed in all the rest of the series, as those of B, C and D. Of these the best growth was found in A and the rest in the order of arrangement of the four salts, as stated above. The growth attained here was better than with single, binary or ternary combinations of the same salts.

For comparison of the results obtained by other investigators on analogous works a few cases may be cited below :

(a) Bacteria.—Lipman[89] with soil bacteria found that the combination of .35 M 100 cc NaCl + 10 cc $MgCl_2$ + 2 cc $CaCl_2$ gives better results in ammonification than any combination of two or three salts.

(b) Plants.—Osterhaut[65,79] on plants has shown that the combination of the four chlorides at a certain molecular concentration gives the best growth of the organism. For instance, with algae he found that the addition of $MgCl_2$ in a combination of NaCl + KCl + $CaCl_2$ enables the plants to live longer than a mixture of three salts. This shows the antagonism of the four salts in combination. The same was true with other plants.

(c) Animals.—Loeb[41] with sea urchins found that the organism does not form any skeleton when treated with a combination of 95 cc $\frac{5}{8}$ n NaCl + 2 cc $\frac{5}{8}$ n $CaCl_2$ + 1 cc $\frac{5}{8}$ n KCl. Even the addition of 1 cc $\frac{10}{8}$ n Na_2CO_3 does not effect the organism. But, when 1 cc $\frac{10}{8}$ n $MgCl_2$ and 1 cc $\frac{10}{8}$ n Na_2CO_3 are added the formation of skeleton ensues in the normal way. This shows the effects of Mg ions on the other metallic ions in

combination. The same investigator[69] found with a jelley fish (Polyorchis) that the spontaneous contraction of the organism continues indefinitely in a mixture of these four salts at certain molecular concentration.

Ostwald[58] with Gammarus found that a mixture of $NaCl + KCl + CaCl_2 + MgCl_2$ becomes more toxic, as shown by the duration of life of the organism, than a mixture of $NaCl + CaCl_2 + KCl$, thus showing a lack of antagonism in a four-salt combination.

The experiments with quaternary combinations show that the yeast behaves similar to those observed by Loeb, Osterhaut and Lipman on animals, other plants and bacteria respectively.

Relative antagonism of ternary and quaternary combinations of salts.

In order to shaw the range of antagonism of ternary and quaternary combinations of salts, the curves in Fig. 5a have been plotted on the same principle, as with binary combinations in the previous publication, from the last microscopic counting of each series. For this purpose the average counting of the check flasks* have been taken as the basis from which to estimate the influence of the various salts in combination. The calculation has been made as follows :

Yeast growth in check flask = x
Yeast growth with first two salts = a
Yeast growth with the third salt = b
Yeast growth with the combination of a and b = c

Toxicity – expected = (x-a) + (x-b)
Toxicity – observed = (x-c)
Antagonism of combinations = (x-a) + (x-b) – (x-c)
Antagonism = x + c-a-b

* The check flasks showed an average growth of 13 millions in all the experiments hitherto performed on yeast

Fig. 5a.

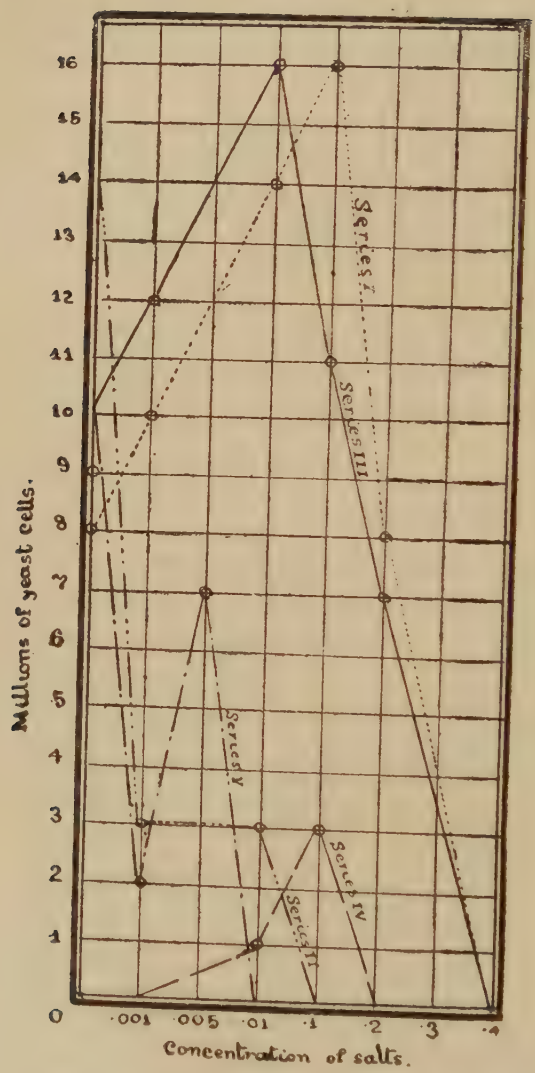

Figure 5a. Curves of yeast growth showing the comparative range of antagonism of ternary and quaternary combinations of salts. The ordinates represent the number of yeast cells in millions and the abscissae, the concentration of salts of different combinations in series 1, 2, 3, 4, and 5. The ordinate at O represents the antagonism of binary combinations.

A PHYSIOLOGICALLY BALANCED NUTRIENT SOLUTION FOR YEAST. (SACCHAROMYCES ELLIPSOIDEUS).

As a result of his experiments on marine animals Loeb concluded that a physiologically balanced solution, in which the toxic effect of one salt is reduced by the addition of others, is essentially needed for the normal development of animals. Osterhaut on plants and Lipman on soil bacteria have corroborated the work of Loeb on this point. The data here will show the results of a similar investigation on yeast to find a physiologically balanced nutrient solution for this organism.

Almost all the authors agree that the chief mineral constituents of a living organism, such as yeast, are at least the four inorganic elements, potassium, calcium, magnesium and sodium. The role played by sulphur in combination with other salts both in fermentation and multiplication of the yeast plant has not been studied yet. It is with a view to find out the influence of sulphur that a series of experiments has been carried on with three sulphate ions. Accordingly, the sulphates chosen for experimental purposes were magnesium sulphate, ammonium sulphate and iron sulphate.

SERIES 6. EXPERIMENTS WITH SULPHATES.

The experiments with sulphates have been carried on in the same way as with the ternary and quaternary series. The quaternary combination of .016 M KCl + .01 M $CaCl_2$ + .001 M NaCl + .005 M $MgCl_2$, that gave the highest growth, was taken at a constant ratio and sulphates, such as magnesium sulphate, ammonium sulphate and iron sulphate, were added in each case separately from .001 M to .1 M. This was primarily arranged to find out their respective influence on yeast.

In all previous experiments the salts were added first to the empty flasks and dilute synthetic solution was put in later by means of a pipette directly on the salts. In all cases where sul-

phates were used, the pouring of the synthetic solution on the salts at once caused a white insoluble compound of calcium sulphate which remained unchanged. Later on, it was found that the addition of salts to the synthetic solution, one at a time, does away with that difficulty, especially at lower concentrations of ·001 M to ·01 M sulphates, while .1 M gave an appreciable amount of precipitate.

It may also be stated that flasks containing .005 M and .01 M sulphates showed an appreciable amount of precipitate after the fermentation was over. Moreover, it was noticed that the same flasks containing .005 M and .01 M sulphates in the synthetic solution with added salts, when allowed to stand for a day or two without being inoculated with yeast, brings down a white precipitate.

A. Magnesium sulphate.

The result of the experiments with magnesium sulphate is shown in the curve a in Fig. 6 and indicates that the best growth obtained in the fourth counting at the quaternary combination was 48 millions, whilst the addition of .005 M $MgSO_4$ increased the growth up to 58 millions, thus showing the influence of the sulphate ion. Besides, the fermentation and multiplication of yeast cells in this case were rather rapid in comparison to the quaternary salts.

It may be added here that in all cases where sulphates were used and the multiplication was rapid, a general decrease of yeast cells was noticed after the fourth counting. This was found mostly where lower concentrations of salts were used. It was not noticed at higher concentrations, where the fermentation and likewise the multiplication was very slow. It is for this reason that the curves in all cases have been drawn from the fourth microscopic counting. In comparison to the effect of magnesiumm

sulphate on yeast and other plants and animals a number of cases may be cited below:

(a) Yeast.— Stern[42] found that sulphur is an essential constituent of yeast nutrient. A portion of sulphates or powdered sulphur was found by him to be invariably reduced to sulphuretted hydrogen. Besides, Hebert[72] experimented on the toxicity of

Fig 6.

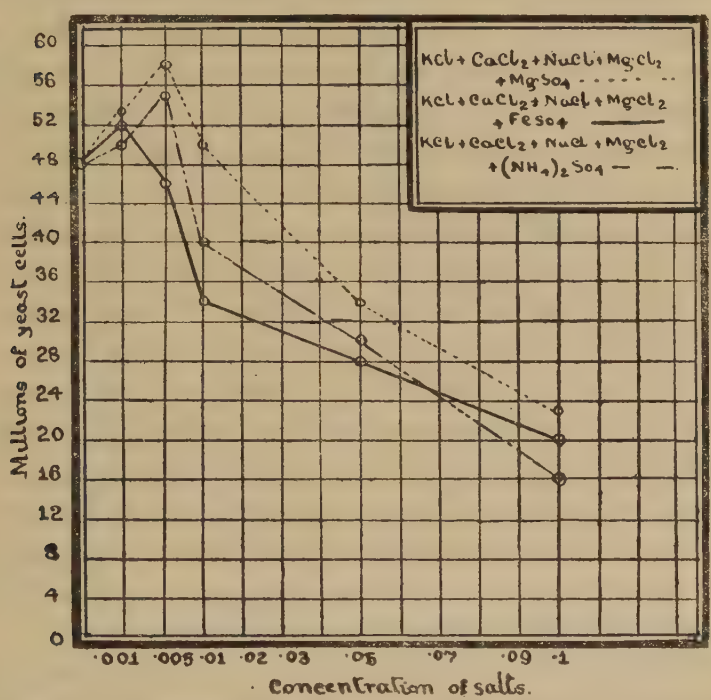

Figure 6. Curves of yeast growth showing the effects of magnesium sulphate, ammonium sulphate and iron sulphate on the quaternary combination of $KCl + CaCl_2 + NaCl + MgCl_2$. The ordinates represent the number of yeast cells in millions and the abscissae, the concentration of salts in combination. The ordinate at O represents the number of yeast cells of the quaternary combination in the ratio of 1·6 : 1 : ·5, while the rest represent the addition of sulphates from ·001 M to ·1 M.

various salts on yeast fungi. He found that in comparison to other metallic salts magnesium sulphate behaves as a non-toxic substance.

(b) Other plants.— In his experiments on the physiologically balanced solutions for plants Osterhaut[65,79] has shown that the mixture of NaCl, KCl, $CaCl_2$, $MgCl_2$ and $MgSO_4$ at certain molecular concentration gives a solution which not only approaches sea water, but is best suited as a physiologically balanced solution for plants. With a young alga (Voucheria sessiles)[64] he showed that it develops and fruits normally in a 3.25 M NaCl to which small quantities of KCl, $CaCl_2$, $MgCl_2$ and $MgSO_4$ have been added, all of which are toxic when used alone. He has also shown with another alga (Enteromorpha hopkirkii)[105] that the combination of .375 M concentration of 1000cc NaCl + 78cc $MgCl_2$ + 38cc $MgSO_4$ + 22cc KCl + 10cc $CaCl_2$ becomes the best medium for normal growth. A similar result was obtained with wheat[79].

(c) Animals.— Ostwald[55] with Gammarus found a similar result in which case the mixture of NaCl, KCl, $CaCl_2$, $MgSO_4$ and $MgCl_2$ at certain molecular concentrations acted as sea water and was best suited to the duration of life of that animal, although the chlorides of these four salts in combination were toxic. It may also be added here that the well-known Ringer's and Vant Hoff's solutions contain these same salts.

All the experiments cited above clearly point to the fact that magnesium sulphate has a stimulating influence on both plants and animals. It plays an important part in the physiology of yeast both by its kations and anions.

B Ammonium Sulphate.

The assimilation of ammonia by plants has been studied by a number of plant physiologists. The results of the experiment with yeast are shown in the curve of Fig. 6. From this curve it is evident that ammonium sulphate has a stimulating influence on

yeast, but not to the same extent as that of magnesium sulphate. For example, the combination in the quaternary salts allowed a growth of 48 millions, while the addition of .005 M ammonium sulphate increased the growth up to 55 millions.

These results with yeast generally coincide with those found in the literature.

(a) Yeast.— Mayer[1] experimented on the nutritive value of a number of nitrogenous substances on yeast. He found that ammonium salts are not very useful in fermentation of the organism. This was corroborated by Ehrlick, * Matthews ** and later by wahl and Hanke. *** Brenner[95] found ammonium sulphate to be assimilated by yeast, but not to the same extent as ammonium tartrate.

Bokorny[114] has shown that the action of ammonia alone, in comparison to other bases, is deliterious to the growth of yeast. Even .5% ammonia was found to prevent the budding of yeast cells, but in combination with sulphates it was found to stimulate the growth. The ammonia apparently combines with yeast, when used alone, and so becomes toxic. Besides, the same author[124] experimented on the effect of ammonium sulphate with dextrose solution to find an increase of 113% of the dried plant.

(b) Other plants.—Lehmann[5] found that buckwheat fails to grow well in ammonium salts, while maize does far better in this form of nitrogen supply than with nitrates during the first peiod of growth. Later on it was observed that the nitrate plants recovered, while ammonium plants became unhealthy. Simlarly, Kellner † showed that rice prefers ammonium salts to nitrates to commence with, but the nitrates are better than ammonium salts for later growth.

*Ehrlick-Der Bierbaur. 8. 145, (161 and 177), 1995.
**Matthews-Jour. Inst. Brewing. P. 369, 1897.
***Wahl and Hanke-Amer. Brewers' Rev. 7, P. 32, 1896.
†Kellner,O.—Landw. Ver.—State., 30 ; 18, 1884.

Frank[12] obtained a satisfactory result in water cultures of beans and sunflowers by the use of ammonium sulphate. Pitsch[13] employed sterilized soils to avoid nitrification by bacteria and applied ammonium sulphate in various plants on humus sand. With oats the result was very satisfactory. Besides, Muntz[14] experimented in a similar way on maize, beans etc. He used sterilized soils in which nitrification of ammonia does not take place and hence concluded that ammonium compounds are directly assimilated by plants. This has also been confirmed later by Pagnoul[16]. Moreover, similar results were obtained by Griffiths[17] on leaves of higher plants in water cultures.

Maze[35] grew maize plants in sterilized water cultures containing ammonium sulphate aud sodium nitrate to find that the whole of ammonia was utilized, while nitrate remained in solution.

Kossowitsch[47] growing peas in sterilized sand cultures found that ammonium sulphate and sodium nitrate are equally suitable sources of nitrogen. This was found to be true by later experiments of Gerlack and Vogel[61]. Besides, Kossowitsch[57] has shown that in ammonium salts the plants take up the basic radicle and leave an acid substratum to the medium. The acidity thus produced will, if it reaches a certain maximum, be injurious to the plants. The results in the case of nitrates instead of sulphates were satisfactory. A similar phenomenon was observed by Prianischnikow[62] with a few higher plants.

Kinoshita[20] and later on Suzuki[24] grew seedlings of various plants in ammonium sulphate and sodium nitrate in order to compare the asparagin produced. It was found that ammonium salts are readily converted into asparagin. They also found that the production of asparagin is promoted by the presence of sugar, while in absence of it or other suitable material ammonia may accumulate in plants and cause injury. Besides, Takabayashi[88] states that ammonium salts bear a noxious effect on phanerogamous

plants, if there be not a sufficient quantity of sugar present in the plant.

Hutchinson and Miller[83] found that agricultural plants (wheat) can produce normal growth when supplied with nitrogen in the form of ammonium salts under conditions which exclude the possibilities of nitrification by bacteria. Later on, the same authors[91] experimented on wheat and peas in which they found that ammonium salts are directly assimilated and it is best applied in the form of nitrate rather than sulphate.

All the experiments, quoted above, show in general a beneficial effect of ammonium sulphate on plants, although in some cases ammonium nitrate showed a better result. However, in the case of yeast a similar stimulating effect was noticed, though not to the same extent as that of magnesium sulphate.

It might be argued here that as the synthetic solution contained ammonia, the addition of ammonium sulphate rather increased the concentration of it above a certain optium, so as to make the medium unfavourable for yeast. But, later experiments with ammonium tartrate will show that this is not the case.

C. Iron Sulphate.

The experiments with iron sulphates was performed in the same way as with the previous ones. The object of using this compound was to determine the influence of both the effect of iron and sulphate at the same time.

The results of the experiment, as is shown in the curve b of Fig. 6, indicate that among the three sulphates used iron sulphate has the least effect on the growth of yeast. For example, the combination of the quaternary salts allowed a growth of 48 millians, while the addition of ·001 M $FeSO^4$ increased the growth up to 53 millions. This increase was perhaps due to the anions of the compound, as has been found in other cases,

while the metallic ions of Fe had little or no effect. This was found to be true when ferric chloride, used in combination with the quaternary salts, gave a negative result.

In comparison to the result obtained with iron sulphate a few cases of general interest may be cited :—

(a) Yeast.—Molisch[22] has found that for normal formation of yeast spores iron in some form or other is necessary, while this was disputed by Wehmer[25]. Besides, Kossowicz[54] has stated the beneficial effects of iron and less of ferric chloride on the growth of yeast.

(b) Other plants.—Boiret and Paturel[18] have found that iron sulphate is beneficial only where there is an excess of lime in the soil. They assumed that its direct influence is to produce gypsum in the soil which prevents the increase of acidity(H_3PO_4) where phosphorous compounds are used. Besides. Katyama with barley found that ·01 % of iron sulphate produced a moderate increase in the crop, but a further increase led to a general decrease in yield. Moreover, Chirikov[109] found a positive result with wheat when iron sulphate was used at lower concentrations, but no definite result was obtained with other plants.

SERIES 7. EXPERIMENTS WITH $KCl + CaCl_2 + MgSO_4$.

In the previous publication on the toxicity of salts the writer has shown that sodium is the most toxic of all the metallic ions experimented on yeast. Aside from this, in all cases where magnesium chloride was used the application of magnesium sulphate was rather a repetition of the same Mg ions. From a consideration of these two points the writer eliminated NaCl and$MgCl_2$ and carried on a series of experiment with a three-salt combination of KCl, $CaCl_2$ and $MgSO_4$. Accordingly, the combination of ·016 M KCl and ·01 M $CaCl_2$, as has been used in ternary combination of salts, was taken at a constant ratio and magnesium

sulphate was added from ·001 M to ·1 M. The object of the experiment was to determine the influence of $MgSO_4$ alone in combination with KCl and $CaCl_2$, as these three metallic ions are of primary importance to yeast growth.

The results in this case was very striking, as is shown in the curve of Fig. 7. From this it is evident that the combination of ·016 M KCl + ·01 M $CaCl_2$ allowed a growth of 30 millions while the addition of ·005 M $MgSO_4$ stimulated the growth to

Fig 7

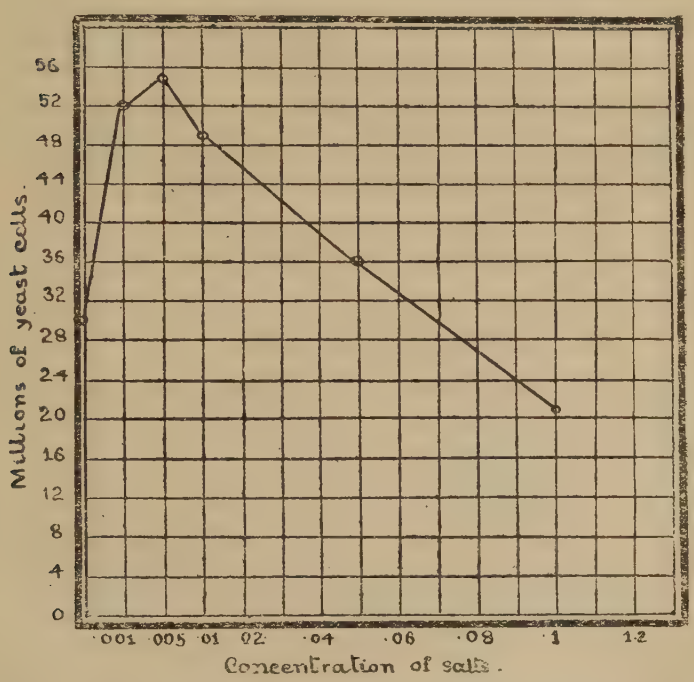

Fig. 7. A curve of yeast growth showing the effect of magnesium sulphate on the binary combination of KCl and $CaCl_2$. The ordinates represent the number of yeast cells in millions and the abscissae, the concentration of salts in combination. The ordinates at O represents the number of yeast cells of the binary combination in the ratio of 1·6 : 1, while the rest represent the addition of magnesium sulphate from ·001 M to ·1M.

55 millions. Besides, the ternary combination of $MgCl_2 + CaCl_2 + KCl$ allowed a growth of 36 millions only. Therefore the growth influenced by magnesium sulphate was 19 milllons more than that of magnesium chloride. Although there is no appreciable difference in growth between this series with that of series 6a, so far as the multiplication of yeast cells are concerned, the rate of fermentation was to a certain extent more rapid in the previous case. Consequently, it is needless to state that the combination in series 6a is physiologically a better solution for yeast than the present one.

Other works indicating the effects of metallic ions used in this experiment on yeast may be cited here for comparison.

Kearney and Cameron[53] experimenting on the resistance of leguminous plants to alkali, found a striking beneficial influence of $MgSO_4$ on the general growth of the plants.

Shive [122] with a three-salt nutrient found that a combination of KH_2PO_4, $Ca(NO_3)_2$, and $MgSO_4$ at certain molecular concentration gives the best yield of top and roots of seedlings. This has been corroborated by the recent work of Mc Call[123] on wheat and soy beans, both in water and sand cultures, Thompson [128] on wheat seedlings, and Shive and Martin [129] on young and matured buckwheat plants.

Besides, investigations on nutrient solutions for higher plants by Knop, Detmer, Pfeffer, Hartwell and Tottingham show that in each case the three metallic ions of K, Ca and Mg have been used in the form of phosphate, nitrate and sulphate, which clearly show the necessity for potassium, calcium and magnesium for the development of seed plants in generel. The yeast is not an exception to this rule.

Series 8. Experiments with Tartaric and Citric Acids and Their Ammonium Compounds.

Analysis of grape juice shows that in addition to the inorganic salts it contains a number of organic acids, such as tartaric acid,

citric acid and malic acid, of which tartaric acid is predominent. As grape juice is a natural physiologically balanced nutrient solution for yeast, the writer has been led to try the influence of organic acids contained in it. Accordingly, the combination of series 6a, *i.e.*, ·016 M KCl + ·01 M $CaCl_2$ + ·001 M NaCl + ·005 M $MgCl_2$ + ·005 M $MgSO_4$, was taken at a constant ratio, and tartaric acid and citric acid were added from ·001 M to ·1 M. As the higher concentrations check the multiplication of the organism owing to the increase of acidity, only the lower concentrations were tried. Besides, the acid salts of ammonium tartrate and ammonium citrate were used in a similar way. The results are shown in the curves of Fig. 8.

It may be added here that a similar decrease in the number of yeast cells, as has been found in series 6, was also noticed here at the fourth microscopic counting, especially in those cases where the fermentation and multiplication of yeast cells were rapid. It is for this reason that the curves have been drawn from the third microscopic counting.

A. Taric acid and citric acid.

From the curves of Fig. 8 it is evident that both the tartaric and citric acids have a stimulating influence on yeast. For example, the combination in series 6a allowed a growth of 50 millions at the third counting, while the addition of ·001 M tartaric acid influenced the growth up to 65 millions. Similarly the combination of ·001 M citric acid increased it up to 63 millions. But, however, a rapid fall in the growth even at the concentration of ·01 M was observed in both cases. Besides a combination of both acids at ·001 M concentration gave a better result than tartaric acid alone.

In comparison to the above results a few cases dealing with the effects of tartaric and citric acids on yeast may be cited below:

Hanson [19] has described in a paper the results obtained by Pasteur in purifying brewer's yeast by growing it in a solution of pure cane sugar acidified with tartaric acid. Duclaux, Volten and

Fig 8

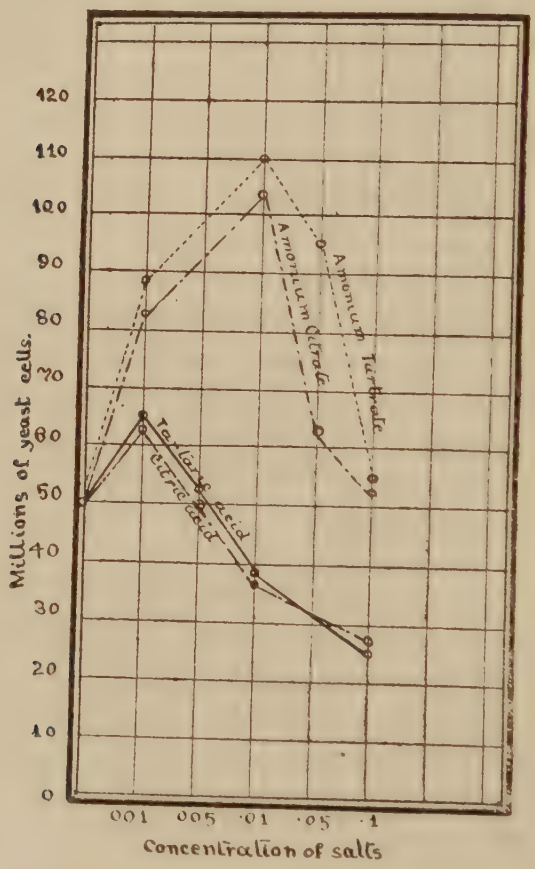

Fig. 8. Curves of yeast growth showing the effects of tartaric acid, citric acid, ammonium tartrate and ammonium citrate on the combination of $KCl + CaCl_2 + NaCl + MgCl_2 + MgSO_4$. The ordinates represent the number of yeast cells in millions and the abscissae, the concentration of salts in combination. The ordinate at O represents the number of yeast cells of the combination of series 6a, while the rest represent the addition of acids and acid salts from ·001 M to ·1 M.

others assert that such a treatment is efficacious to rejuvinate a yeast.

Emmerling[39] has shown that the conversion of malic acid to succinic acid by brewer's yeast is due to bacteria. The same author cited a case in which a similar result was obtained by König to the effect that tartaric acid is largely reduced to succinic acid by some bacteria or yeast.

Schwarz[94] found tartaric actiod be a source of energy to yeast cells. Besides, Karczag[102] experimented on the nature of the effect of tartaric acid on yeast and found that with fermentation the volatile acids are derived from tartaric acid, through malic and glyceric acids as intermediate products.

Ventre[110] studied the fermentation of sugar solutions by different yeasts of varying acidity in media and of the effect of using organic acids to produce initial acidity. He found tartaric acid to remain almost unchanged in original amount, while malic acid was most readily attacked by yeast.

Schukow[29] found citric acid to be more readily consumed by yeast than malic acid and also much less by tartaric acid. Buromsky[118] has rather corroborated the observation of Schukow to the effect that the multiplication of yeast cells is least effected by tartaric acid. But he also found that a small quantity of tartaric acid stimulates the growth of yeast.

The above quoted experiments, except that of Schukow and Ventre, show a general agreement to the present work on yeast. The conflicting results here are justified by the report of Kunz[115] who found tartaric acid in top yeast but not in bottom yeast. The reason for such a peculiar phenomenon of two varieties of yeast in the same culture medium is mainly due to a power of selective absorption of different varieties of yeast plants.

It may be added here that an experiment performed with a combination of three salts of series 7 with the addition of tartaric acid and citric acid gave a similar result.

B. Ammonium Tartrate and Ammonium Citrate.

Pasteur maintained that the yeast plant during fermentation can derive from ammonical salts the nitrogen necessary for its life. This assertion was strongly objected to by Liebig, but was later on proved by Greissmayer[2], thus corroborating the work of Pasteur. Besides, in seeking an artificial nutrient solution for yeast, Pasteur*found ammonium tartrate to be the best material for the purpose. It is with a view to determine the influence of both ammonium tartrate and ammonium citrate that the writer has used these salts to find out their respective influence on the organism.

From the curves in Fig. 8 it is evident that the combination in series 6a allowed a growth of 50 millions, while the addition of ·01 M ammonium tartrate raises the growth to 110 millions. Even the addition of ·025 M ammonium tartrate approaches the same. Similarly the growth obtained by ammonium citrate at the same concentration stimulated the growth to 104 millions.

In order to compare the results of the above experiments on yeast only one case may be cited below :

Brenner[95] found that ammonium tartrate is one of the most suitable forms for nitrogen assimilation in Aspergillus niger, comparable to the effect of asparagin. He also found that the same organism assimilates ammonium citrate better than ammonium sulphate.

It may be added here that an experiment performed with ammonium tartrate combination with the salts of series 7 produced a similar result both in fermentation and multiplication of yeast cells.

The growth of yeast obtained with ammonium tartrate has been found to be the best in all the mixtures of organic and inorganic salts hitherto tested. This mixture allowed a growth which is comparable to a certain extent with the multiplication of

* Pasteur, L. Annal d. Chim et d Physique, 1862, 3 ser., Lxiv, 1.

yeast cells obtained by dilute grape juice or malt-wort. But it is to be noted that the growth obtained either with grape juice or malt-wort, as will be shown later, is very rapid in comparison to the artificial culture of series 8 (ammonium tartrate). For, with a natural medium the fermentation becomes complete in three or four days, while in the synthetic solution, with the addition of nutrient salts, the fermentation continues for six or seven days.

The experiments here show that so far as the artificial culture is concerned the mixture of ·016 M KCl + ·01 M $CaCl_2$ + ·001M $NaCl$ + ·005 M $MgCl_2$ + ·005 M $MgSO_4$ + ·01 M $(NH_4)_2C_4H_4O_6$ (ammonium tartrate) is a physiologically balanced nutrient solution for yeast (S. ellipsoideus). Besides, the three-salt combination of series 7, namely, ·016 M KCl + ·01 M $CaCl_2$ + ·005 M $MgSO_4$ + ·01 M $(NH_4)_4C_4H_4O_6$ is also a physiologically balanced nutrient solution for yeastt, but not to the same extent as that of the former. Moreover, the nature of the physiological solution obtained here with yeast is identical with that found by Osterhaut, Tottingham and Shive with plants, Loeb and Ostwald with animals and Lipman with soil bacteria.

It has been stated before that after the fermentation sets in a white precipitate of $CaSO_4$ occures in the culture flasks containing the added salts. In order to avoid this unused calcium and and sulphate ions, an experiment was tried with a lesser amount of $CaCl_2$ and $MgSO_4$. The result was rather unsatisfactory. Why such an excess of these two salts is needed by yeast at the outset of fermentation and is separated later from the medium, as fermentation proceeds, is not known. Crone [63] met with a phenomenon in his work with higher plants. He concluded that the assumption that the constituents of a nutritive medium should all be in solution is incorrect. He observed that good results were obtained where ferrous phosphate and tri-basic calcium phosphate were used in a solution having an appreciable precipitation.

Degeneration of Yeast Cells.

In yeast cultures of series 6, 7, and 8 the combinations that were best suited for the growth of the organism, showed a general decrease in number of yeast cells after the fermentation was complete. The cultures, in which the concentration of salts was rather high and fermentation was accordingly slow, did not show such a decrease. Jacobsen[9] reported that brewer's yeast degenerates if no change is made. However, in those cases where the number of yeast cells actually decreased, after fermentation was over, the cells were found to be in good condition under the microscope.

Whatever relation there exists between the farmented medium and the living yeast cells in it, it was found by titration of the fermented liquid with a N/10 NaOH (as tartaric acid) that the acidity of the medium increases very markedly with fermentation. This acidity decreases with the increasing concentration of salts or rather with the decreasing fermentative capacity of the medium. But, in any case the end product of the percentages of acidity was almost fixed.

It has been found by titration even two months after fermentation was over that the acidity of the fermented medium does not increase appreciably. The accompanying table shows the percentages of acidity in a series of experimental flasks of series 8 (ammonium tartrate) after the fermentation was complete. The average acidity of the synthetic medium ranged from ·2 to ·3%.

Percentage of Acidity After Fermentation.

	Number of days.			
	15	30	45	60
Culture 1	1·01	1·07	1·00	·97
„ 2	1·30	1·50	1·46	1·40
„ 3	1·30	1·40	1·55	1·46
„ 4	1·10	1·97	1·24	1·24
„ 5	1·00	1 08	1·10	1·20

From the table it is evident that the acidity of yeast juice is rather uniform when fermentation is complete, although a slight increase is shown in cultures Nos. 2 and 3, which were best suited for the growth of the organism. This slight increase in acidity was observed up to the third titration in all cases, while the fourth titration showed a slight decrease in acidity in the first three cultures where a comparatively lower concentration of salts was used. Whether the production of such an excess of acidity is the cause of degeneration of yeast cells is not known.

Kayser and Demolon reported that after fermentation was complete the yeast cells behave as physiologically normal cells in which the intramolecular respiration is replaced by normal respiration during which the yeast utilizes the organic acids as nutritive material.

In regard to the theory of acid poisoning in herbivorous and carnivorous animals it has been found by a number of animal physiologists. especially by Winterberg[36] that both classes of animals secrete some basic substance (ammonia) to neutralise the acid of which the carnivorous do more than the herbivorous animals. The yeast plant does not seem to have any corresponding process by which a base is secreted to neutralise the acidity in the medium, although some of the algae have been reported to render the water alkaline in which they grow*.

Furthermore, it has been found by Harvey[127] that in the injury of cabbage plants by frost the main cause of deterioration of the cells exposed is due to the increase of acidity caused by loss of water from the cells. The higher concentration of H-ions results in the precipitation of proteins and ultimately causes the death of the cells. Whether such a phenomenon occurs in the yeast is not known.

An experiment was performed in a few fermented flasks from

* Bayley Balfour, "Nature", Sec. 1, 1886.

series 8 (ammonium tartrate) in which ammonium hydroxide was used to partially neutralise the acidity of the media to compare with similar untreated flasks. Microscopic counting before and after refermentation showed that in the neutralized flasks the growth of the organism was 25% to 30% more than the untreated ones. Pure sterilized cane sugar syrup was used in both cases for refermentation. Even the addition of a small quantity of ammonium chloride to the untreated flasks did not show any appreciable improvement in multiplication of the organism. This shows the toxicity of excess acidity in the fermented medium and that it is connected with the degeneration of yeast cells in artificial media.

In regard to the nature of acid and its effect on yeast fermentation a number of cases may be cited :

Bungener[11] showed that a yeast which has been repeatedly used for fermenting purpose becomes unfit for further use after several generations. A degeneration of yeast cells was found to occur in many cases of which no definite cause was known. He assumed that the presence of lactic or acetic acid ferments together with the composition of wort, the quantities of fermentable sugars, extracts, salts and nitrogenous constituents it contains, have a great deal to do with it.

Gerard and Darexy[31] found that the fatty matter obtained from yeast contains stearic and palmitic acids together with a small amount of butyric acid. In their opinion these acids occur to a certain extent as their glyceric salts and also probably in the pure form.

Henneberg[67] experimented on yeast to determine the effect of formic acid. He found that the yeast failed to develop in a 2% solution of formic acid, while bacteria and fungi are more susceptible to the same even at less concentration. The same investigator[88] found that in acid solution the yeast dies more rapidly than in alkaline solution.

Drable and Scott [77] found that at the concentration of .1 M HCl and HNO_3 in a beer-wort medium both fermentation and multiplication of yeast cells are arrested and that a general shrinkage occurs in all the cells and ultimate death. Besides, Kisch[101] found that the concentration of H ion higher than N/9 in a water medium permanently injures the cells in a short time. Osterwalder[97] analysed the fermented yeast juice to find that the amount of volatile acids after fermentation rises for four or five months and increases up to 1.8%. The writer determined the volatile acids of a few flasks in series 8 (ammonium tartrate) both after fermentation was over and after a period of 60 days. The volatile acid was found to average 1 to 1.3% respectively, while the total acid showed a difference of .24% only.

Franzen and stepphun[98] reported the production of formic acid during fermentation. They asserted that the production and presence of formic acid is due to an enzymic action that is closely related to the actual decomposition of sugar to alcohol and carbon dioxide.

Fernbach [111] found that the acidification of yeast is subject to the acidity of the medium favouring high or low production. The writer has found that a culture of grape juice containing an initial acidity of .8% showed increase of only 1.4% after fermentation was over. However, the increase was not so marked as was found with the synthetic medium which was only slightly acid. A higher percentage of acidity in the synthetic solution to begin with, as is found naturally in grape juice, inhabits the growth of organism entirely. This shows that the acidity of grape juice is toxic to yeast, but it is the presence of other inorganic and organic substances that reduce or neutralize the toxicity of acid in the juice. Besides, it has been shown by Moufang[120] that the acidity of the medium largely depends on the kind of sugar fermented and increases with temperature.

The question may arise whether the accumulation of alcohol causes the degeneration of yeast cells after fermentation is complete. Accordingly, a few flasks were analysed (volumetric) to determine the percentage of alcohol in the fermented flasks in series 8 (ammonium tartrate) which was found to range from 2.3 to 2.5%.

Hayduck[5] found that 5 % alcohol does not affect the yeast. He* also reported that in the course of fermentation fusel oil develops which impedes the process of multiplication of yeast cells, although the fermentative power remains. On the other hand, Delbruck[8] found that 5% to 7% alcohol in a "mash material" prevents the growth of yeast cells, although this does not destroy the fermentative capacity of the organism. Besides, Lindner and Cziser[107] found that 2% to 6% alcohol is a good source of food for yeast.

In all cases where the sulphate ions were used a very distinct odour of hydrogen sulphate was noticed. The poisnous action of this gas is very well known in animals.† How far the accumulation of this gas in culture flasks affect the yeast is not known.

Schander[56] has found that yeast produces hydrogen sulphide from free sulphur and sulphur compounds. He found the activity of yeast to be stimulated by sulphates and usually by free sulphur. Besides, Chowrenko[100] states that certain yeasts have the property of hydrogenation of sulphur during fermentation. He found that an increase of sugar content leads to greater production of hydrogen sulphide. Later on, Sasaki and Otsuka[104] reported that many bacteria produce hydrogen sulphide from cystin and other sulphur compounds in culture media.

To summarise the above discussion on the degeneration of of yeast cells it may be stated that the production of alcohol (probably fusel oil too) and hydrogen sulphide are rather negligible

*Hayduck, M.—Jour. Soc. Chem. Ind., Vol. II, p. 323, 1890.

†Newth's Inorganic Chemistry, p. 369.

in such an artificial culture medium and so likewise their respective poisnous effects on yeast. It is evident therefore that the production of an excess of acidity is the direct cause of degeneration of yeast cells.

Effects of Some Organic Substances on Yeast.

The organic substances that have been used in previous experiments are hydrolised cane sugar, the hydroxy acids, such as tartaric and citric, and the acid salts, ammonium tartrate and ammonium citrate. Attempts will be made here to show the respective effects of glucoside, such as amygdalin; tannin, such as tannic acid; nitrogen bases, such as methylamine, quinine and strychnine; amino acids, such as aspartic and glutamic acids; amides, such as asparagin and glutamin; and lastly peptone. Most of these organic substances are found to occur in grape juice. It may be added here that the three-salt combination of series 7 has been used here as a constant ratio and the different organic salts have been added according to their respective molecular concentrations, except that of tannin and peptone, which were added by their respective weight.

Series 9. Experiments with Glucoside and Tannin.

The experiments with glucoside and tannin were carried on in the same way as with previous series. For this purpose amygdalin (Merck's) and tannic acid (Kauffman Latimar & Co.) were used. The flasks were arranged as before and the combination of salts in series 7 was taken at a constant ratio. Accordingly, the mixture of .016 M $KCl + .01$ M $CaCl_2 + .005$ M $MgSo_4$ was added to the flasks containing the synthetic solution and amygdalin was added at concentrations of .001 M and .01 M. Similarly, tannin was added to the abave combination of salts from ·01 gram to .5 gram. The results of experiments in both cases are shown in the curves of Fig. 9.

A. Amygdalin.

From the curve a in Fig. 9 it is evident that amygdalin has little or no effect on yeast. For instance, the combination of .016 M KCl + .01 M $CaCl_2$ + .005 M $MgSO_4$ allowed a growth of 52 millions at the third counting, while the addition of .001 M amygdalin showed a growth of 54 millions. Here the increase of two millions is negligible. Besides, the same combination of salts with .01 M amygdalin showed a decided toxic effect.

In this connection a few cases dealing with amygdalin on other plants may be cited for comparison :

Concerning the decomposition of amygdalin by micro-organisms Fermi and Montesano[23] state that molds and bacteria have the power to break down amygdalin and derive their nutrient from it. Puriewitsch[37] found that the mycelium of various fungi, such as Aspergillus and Penicillium, has the power of decomposing glucosides to break them into glucose and some benzol derivatives. This glucose was found to be taken up by the mycelium, while the other products were either partially absorbed or left in the culture medium. The same author also reported the decomposition of amygdalin by various molds and its assimilation by the organisms with the formation of ammonia. Brunstein[49] confirmed these results with amygdalin using the same organisms.

Contrary to the above results Cook and Taubenhaus[56] obtained a negative result with amygdalin on parasitic fungi that cause diseases of cultivated plants.

B. Tannic Acid.

From the curve b in Fig. 9 it is evident that tannic acid like amygdalin has little influence on yeast, although the addition of ·01 gram shows a littte better growth than amygdalin. For example, the combination of series[7] allowed a growth of 52 millions, while the add ition of .01 gram of tannic acid raised it up to 57 millions.

However, the growth of yeast dropped down very markedly as the concentration was raised to .05 gram.

Fig 9.

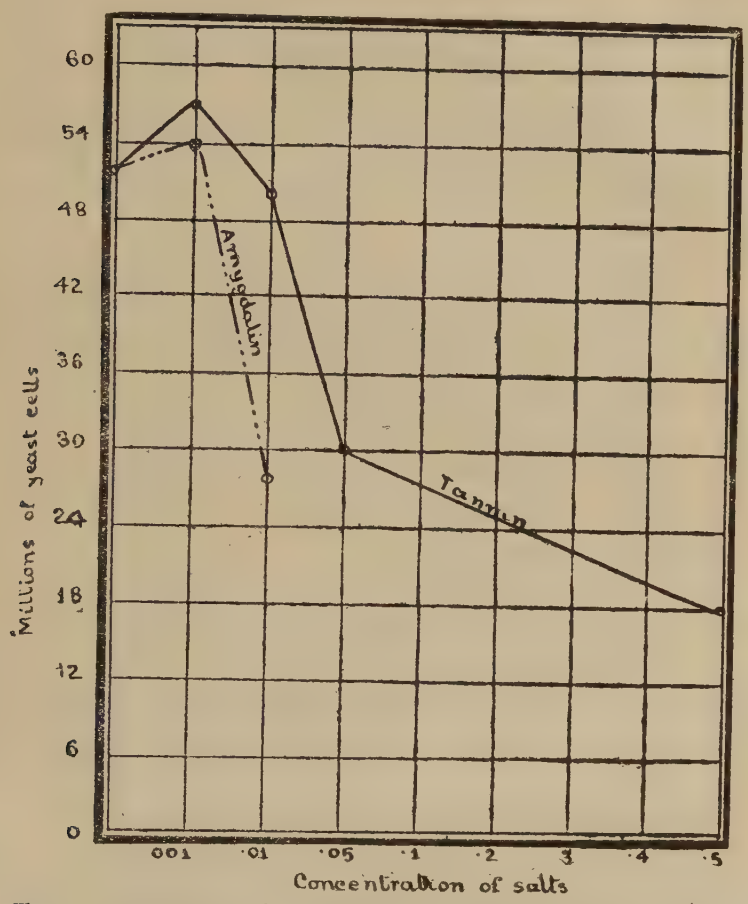

Fig. 9. Curves of yeast growth showing the effects of amygdalin and tannin with the three-salt combination of $KCl + CaCl_2 + MgSO_4$. The ordinates represent the number of yeast cells in millions and the abscissae, the concentration of salts in combination. The ordinate at O represents the number of yeast cells of three salt combination in the ratio of 1.6 : 1 : .5, while the rest represent the addition of amygdalin at .001 M and .01 M, and tannin from .01 gram to .5 gram.

Other experiments dealing with the effect of tannin on yeast and other plants may be cited :

Stern[12] experimented on the nutritive value of tannin for yeast. He found that even the use of small percentages reduced the number of yeast cells in culture media. Besides, Rosenstiehl[51] found that the yeast combines and withdraws tannin from a solution, as a result of which the fermentative power of the organism is reduced and finally becomes nil, although the power of reproduction may still persist.

Cook and Taubenhaus[96] in their experiments on the different types of culture media containing various amount of tannin innoculated with parasitic and saprophytic fungi, found a general inhibitory effect on the organism, except when small amounts were used, but in any case .1 % to .4 % of tannin was definitely found to be toxic. Besides, the toxicity of tannic acid has been shown by Knudson[112] to a number of parasitic and saprophitic fungi even at relatively low concentrations.

All these above citations in regard to amygdalin and tannin clearly signify that they have little or no influence on yeast or other plants. Although they may not be toxic when used in a small quantity, and instead may increase the growth to a certain extent, yet at higher concentrations they are always injurious to plant life.

Series 10. Experiments with Nitrogen Bases.

In this series of experiments with nitrogen bases the writer used one member of the amine group and two alkaloids. A 30 % solution of methylamine and Baker's quinine and strychnine were used for this purpose.

The combination in series 7, *viz.*, .016 M KCl + .01 M $CaCl_2$ + .005 M $MgSO_4$, was used at a constant ratio and the nitrogen bases were added according to their respective molecular concentrations. Accordingly, a calculated amount of methylamine solution was added from .001 M to .1 M, while both quinine and

strychnine were added at .001 M and .01 M concentrations. The results of the experiments are shown in the curves of Fig. 10.

A Methylamine.

From the curve a of Fig. 10 it is evident that methylamine has a stimulating effect on yeast. For example, the combination of series 7 allowed a growth of 52 millions, while the addition of .01 M Methylamine raised the growth up to 86 millions. Even

Fig 10.

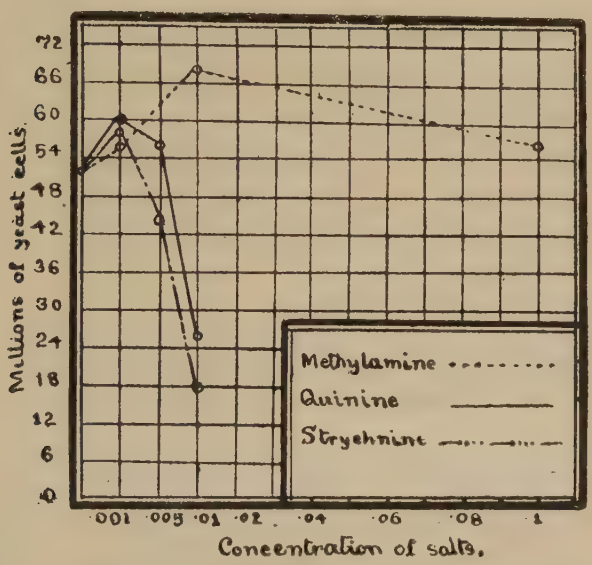

Fig. 10. Curves of yeast growth showing the effects of methylamine, quinine and strychnine with the three-salt combination, $KCl + CaCl_2 + MgSO_4$. The ordinates represent the number of yeast cells in millions and the abscissae, the concentration of salts in combination. The ordinate at O represents the number of yeast cells of the three-salt combination, while the rest represent the addition of methylamine from .001 M to .1 M, and quinine and strychnine from .001 M to .01 M.

the addition of .1 M did not show any toxic effect. The growth here was, however, not so well defined as that found by aspartic acid.

Lutz[33] on sterilized soil found that phaenerogamous plants can obtain their nitrogen from amines of low molecular weight, such as methylamine, ethylamine etc. Later the same investigator on flowering plants, algae and fungi found that these plants can take up the nitrogen necessary for their growth from salts containing amino compounds, Moreover, Ehrlich and Pitschimuka[99] reported that the action of yeast on amino compounds has been extended to primary amines, which are converted into corresponding alcohol with the liberation of ammonia. In their opinion amines are intermediate compounds in the formation of alcohol from amino acids by yeast.

A contrary result was obtained by Mollard[85] on radishes in which methylamine was found to show an inhibitory effect, while ammonium chloride and calcium nitrate showed a stimulating influence.

All the experiments, quoted above, with the single exception of Mollard, show that methylamine has a stimulating effect on yeast, as well as on other plants.

B. Quinine and Strychnine.

From the curve in Fig. 10 it is evident that the alkaloids are usually toxic substances to yeast, although there is a slight stimulating effect at lower concentrations. For example, the combination of series 7 allowed a growth of 52 millions, while the addition of .001 M quinine and strychnine raised the growth up to 60 and 58 millions respectively. This increase is undoutedly due to the NH_2 group of the alkaloid compounds. But, the rapid fall in growth even at .005 M in both cases shows that they are rather toxic to yeast, although at lower concentrations they influence the growth to a certain extent.

Weyl[7] with Elodea Canadensis found that strychnine nitrate causes yellowing of the leaves. It hinders expiration of oxygen very markedly. The same author found that the application of morphine hydrochloride does not effect plants in the same way and so is less toxic. On the other hand, Mosso[21] found that the germination of the seeds of phaseolus multiformis is hastened by .05% strychnine, while higher concentrations are injurious. Besides, Otto[27] found that the addition of strychnine phosphate was unfavourable to the growth of four weeks' old bean plants grown in sand culture and humus soil.

Lutz[33] experimented with phaenerogams under sterilised condition and found that they can obtain their nitrogen from amines, but, when ammonium salts and alkaloids are substituted no beneficial result occurs. The same investigator also found later that the flowering plants, algae and fungi can partially assimilate the nitrogen from the alkaloids, if there be any assimilable nitrogen to begin with.

Morgulis[81] found strychnine to exert a stimulating influence on the regeneration especially at lower concentrations. Amongst the algae Come're* found that Ulothrix subtilis and Spyrogyra crassa grown under aseptic conditions and in a solution free from nitrates, could make use of certain alkaloids as a source of nitrogen. Moreover, Sigmund[116] found strychnine as an active substance to influence the germination of seeds at the concentration of .02 M to .04 M per litre of water.

A negative result was obtained by Morkowine[48] on the leaves of Vicia faba which yellowed on the application of alkaloids. Besides, Otto and Kooper[89] found that the presence of alkaloids in solution retards the germination and growth of plants leading to the development of abnormalities.

All these experiments show that the alkaloids are generally toxic to plants although they are found to stimulate the growth

*Come re—Bull. Soc., Bot., France 57, P. 277,

when used at low concentrations. The yeast here agrees in its behaviour with those of higher and lower plants.

Series 11. Expeiments with amino acids, amides, and peptones.

In this series the writer attempted to determine the influence of amino acids, amides and and peptones. Two amino acids, viz., aspartic acid and glutamic acid and two amides, viz. , asparagin and glutamin, were tried. Of these amino-compounds aspartic acid and asparagin were Marck's reagents, while glutamic acid and glutamin were used from laboratory preparations.

The method adopted was practically the same as in previous series. Accordingly, the combination in series 7 was taken at a constant ratio and the amino acids and amides were taken from .001 M to .1 M, while peptone (Witte's) was taken at .1 and .5 gram by weight. The curves in Fig. 11 show the results obtained in all cases.

A. Amino acids

From the curves of Fig. 11a it is evident that the amino acids such as aspartic and glutamic acids, have a decided stimulating influence on yeast. For example, the combination in series 7 allowed a growth of 52 millions, while the addition of .005 M aspartic acid and glutamic acid increased the growth up to 87 and 76 millions respectively. The increased concentration of both aspartic acid and glutamic acid. even at .01 M acted toxically to a certain extent. This toxicity is perhaps due to the acid group (COOH) which increased the acidity of the synthetic medium. Both the aspartic acid and glutamic acid are almost similar in their behaviour to the organism of which the former is more favorable than the latter.

In comparison to the results obtained above a number of

reports by various investigators on yeast and other plants may be cited below:

(a) Aspartic acid.

(b) Yeast:—Pringsheim [72] observed that aspartic acid is a suitable nitrogenous compound for fermentation by yeast. The same investigator [76] in his work on the nitrogenous nutrition of yeast found that no fermentation occurs without the formation of amino acids in the fermenting medium. Besides, Neubauer and Fromherz [92] have shown a possible analogy between the conversion of amino acids into alcohol by means of yeast and into fatty acids in mammalian organism. In each case it is considered that

Fig 11a.

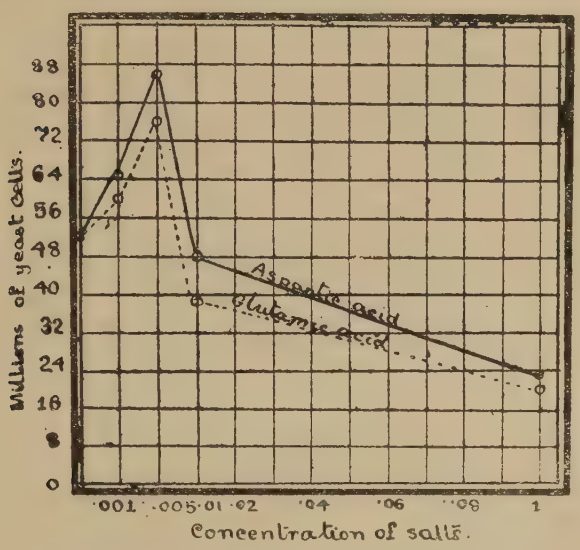

Fig. 11a. Curves of yeast growth showing the effects of aspartic acid and glutamic acid with the three-salt combination of series 7. The ordinates represent the number of yeast cells in millions and the abscissae, the concentration of salts. The ordinate at O represents the number of yeast cells of the three-salt combination, while the rest represent the addition of amino acids from .001 M to .1 M.

the ketonic acid (R—CO—COOH) is the intermediate product.

Waterman [119] experimented on the assimilation of nitrogen by yeast from aspartic acid which gave a very satisfactory result. Besides, Bokorny[124] with yeast showed aspartic acid to allow an increase of 61. 3. % by weight of the dried yeast after complete fermentation.

(2) Higher plants:—Prianischnikoff and Lebedeff [32] obtained a negative result with aspartic acid on a number of plants, but previous to their work Laurent[15] showed that when sugar is supplied, the plants can assimilate 1 % solution of aspartic acid, otherwise, a toxic effect results. Besides, Brown [71] found aspartic acid to be easily assimilated by excised barley embryos.

According to Schreiner et al[75] wheat plants are killed by .05 % aspartic acid, whilst .01 % is not toxic. Later Schreiner and Reed [82] found with wheat seedlings that amino acid compounds display a relatively low toxicity in comparison to other nitrogenous organic substances, although some of them seemed to serve as nutrients to plants. Moreover, Mollard [85] found aspartic acid to be rapidly assimilated by various kinds of higher plants.

(b) Glutamic acid.

(1) Yeast:—The only experiment with glutamic acid on yeast is that of Ehrlich [78] who found that the production of succinic acid during alcoholic fermentation is derived from glutamic acid after gradual interchanges of other acids.

(2) Higher plants:—The only experiment with glutamic acid on higher plants is that of Brown [71] whose results with excised barley embryos showed that the effect of glutamic acid in the assimilation of nitrogen was practically the same as with tartaric acid.

It may also be added here that a series of experiments were performed with tyrosin. The result ahowed a similar growth as that of glutamic acid. It seems that tyrosin is a little more toxic than glutamic acids at higher concentrations.

All the experiments quoted above on yeast and other plants aspartic acid and glutamic acid show that the present results with yeast are in genral agreement with the work of other investigators.

B. Amides.

From the curves of Fig. 11b it is evident that amides have a striking influence on yeast. For example, the combination in series 7 allowed a growth of 52 millions, while the addition of .05 M aspargin and .01 M glutamin stimulated the growth to 142 and 125 millions respectively. Besides .1 M asparagin did not show toxicity, while glutamin at the same concentration showed slight toxicity.

(a) Asparagin.

(1) Yeast:—Almost all the early investigations of Mayer [1] Birner, [6] Hayduck [4] and Stern [42] show asparagin to be a suitable substance for alcholic fermentation and nutrition of yeast cells Pringsheim [76] stated that aspargin is the most important factor to bring about the quickest fermentation and the best nitrogenous metabolism. But when asparagin and ammonium sulphate were used in combination, the fermentation and likewise the multiplication of the organism was found to be decreased with the increase in concentration. Besides, Waterman [119] showed that asparagin is easily assimitated by yeast in artificial culture media. Furthermore, as a result of his experiments on nitrogenous compounds Bokorny [124] found asparagin to increase the growth of yeast by 103. 7 % of the dry weight.

(2) Other plants :—Baessler[10] showed that asparagin is readily assimtlated by maize plants provided that the roots do

not remain too long in the solution, otherwise they are liable to be injured by the action of decomposition producta. In his experiments with green plants on the assimilation of asparagin Loew[26] found that it is the most important substance in the production of protein witnin the plant cells. Besides, Klebs* has shown with algae and fungi that 1% solution of asparagin inhibits the growth of Zoospore formation in Conferva minor, while lesser concentration is suitable for the same purpose. Moreover, Nakamura[30] used 1 % solution and found that barley plants grow better than at the same concentration of ammonium succinate.

Hansteen[38] with Lemna has shown that when glucose is present in the medium asparagin is converted into proteins in the leaves of plants growing in the absence of light. This does not occur when sucrose is used.

Brown[71] showed that excised barley embroys produced as much dry matter with asparagin as with nitrate of sodium, although in the former case the percentage of nitrogen was found to be higher. In his experiments on wheat seedlings Schreiner et al[75] found that .1% asparagin was beneficial to the plants, while increasing concentrations showed a toxic effect. Besides, Mollard[85] found asparagin to be easily assimilated by higher plants at the same concentration.

(b). Glutamin.

In his experiments with Lemna Hansteen[38] found that in presence of glucose glutamin is converted into proteins by leaves growing in absence of light, but not when sucrose is employed. The result accords with that obtained with asparagin, whilst with glycin the presence of sucrose and not glucose was found to be essential.

* Klebs, G., Die Bedingungen der Fortpflanzen bei einigen Algen uud Pilzen. Jena.

Hutchinson and Miller[91] experimented on the assimilation of organic substances by corn, beans and other plants to find that glutamin like asparagin is easily assimilated by the plants.

In his discussion on the synthesis and breaking down of organie nitrogen in plants, Schulze[70] pointed out the beneficial effects of glutamin and other nitrogenous comdounds.

The experiments cited above concerning asparagin and glutamin on yeast and other plants, show that these two amino

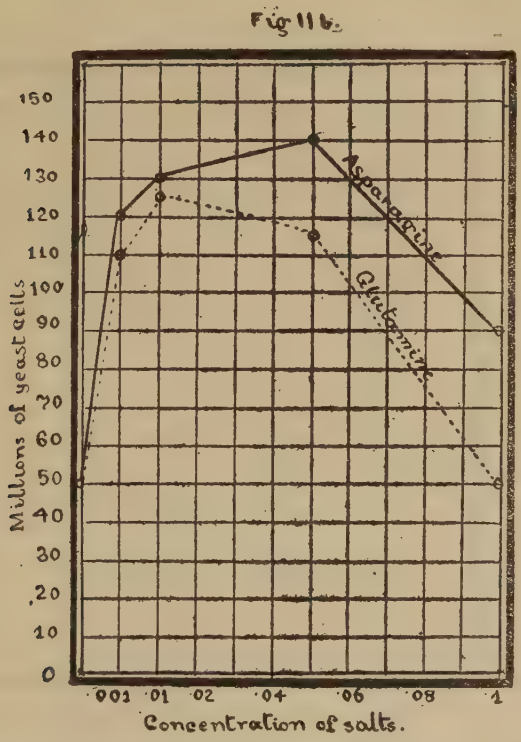

Fig. 11b. Curves of yeast growth showing the effects of asparagin and glutamin with the three-salt combination of series 7. The ordinates represent the number of yeast cells in millions and the abscissae, the concentrations of salts in combination. The ordinate at O represents the number of yeast cells of the three-salt combination, while the rest represent the addition of amides from .001 M to .1 M.

compounds are universally beneficial to plants aud are very easily assimilated by yeast, The beneficial effect of asparagin is more pronounced than that of glutamin. The writer's experiments here show similar results as those of other workers on yeast and other plants.

C. Peptone.

The results of the experiments with peptone are shown in the curve of Fig. 18c. From this curve it is very evident that

Fig. 11c

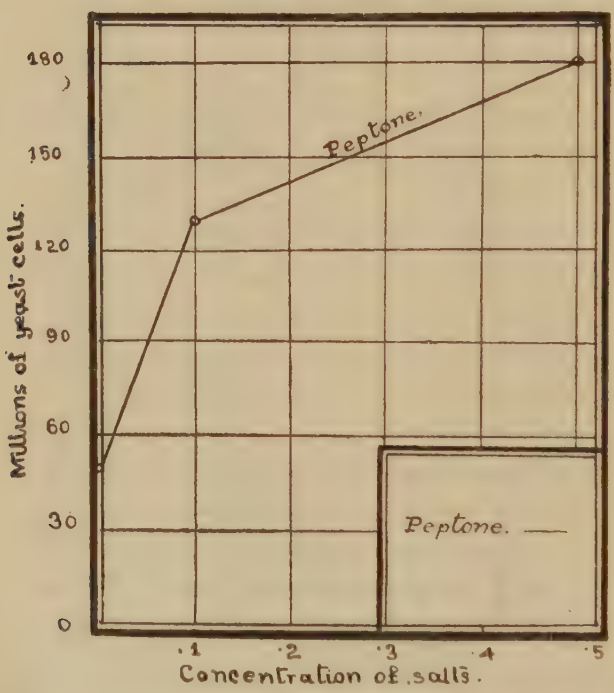

Fig. 11c. Curve of yeast growth showing the effects of peptone with the three-salt combination of series 7. The ordinate represents the number of yeast cells in millions and the abscissa, the concentration of salts in combination. The ordinate at O represents the number of yeast cells of the three-salt combination, while the rest represent the addition of peptone from .1 gram to 5. gram.

peptone has a very striking influence on fermentation and multiplication of yeast cells. For example, the combination of series 7 allowed a growth of 52 millions, while the addition of .5 gram peptone stimulated the growth to 180 millions.

The results obtained by other experimenters using peptone with yeast, other plants and bacteria are as follows:

Mayer[1] found that diffusible peptones are very suitable for yeast fermentation. This has been coroborated by the work of Matthews* Ehrlich, * * and Wahl and Hanke. *** The latter investigators found that only a small portion of peptone is assimilated by yeast.

Lange [41] in a comparative study on the effects of peptone and asparagin on the nutrition of yeast could not detect any difference in the rate of fermentation between two organic substances employed in the organism. Pringsheim[76] found that if peptone is used in increasing concentration it leads to increasing growth and fermentative activity. Lastly, Bokorny[124] has shown that the use of peptone alone increased the growth by about 152%, while with sucrose in combination the growth was increased only 117% by weight of the dried yeast after fermentation was complete.

(2) Other plants:—Effrot[59] experimented on the influence of peptone on the germination of seeds and found a stimulating effect in all cases. Hutchinson and Miller[91] reported satisfactory results by the use of peptone on wheat plants.

Among the lower organisms Fousek[108] determined the assimilation of nitrogen by Streptothrix in the soil with peptone and found that it is easily decomposed by the organism with liberation of ammonia. Besides, Lipman[87] showed that the ammonification of soil bacteria reaches its maximum in artificial media when peptone is used. Moreover, Kunkel[121] found that the use of

* Loc. Cit.

** Ibid.

*** Ibid.

peptone removes the toxicity of inorganic salts on Monilia sitophila.

All these experiments mentioned above shows that peptone has a stimulating effect on plants in general, and is most pronounced on lower plants. The results of the experiments on yeast agrees with those of other investigators on yeast, other plants and bacteria.

The following is a list of percentages of growth obtained with the addition of inorganic and organic salts in the synthetic medium on yeast. In all cases the growth of the synthetic solution alone has been taken as the standard from which to count the percentages of growth in all the combinations showing the percentages of net increase therein.

Combination with synthetic solution.	Maximum growth.	Percentage of actual growth.	Percentage of net increase.
1. Synthetic solution	13	...	...
2. KCl	26	100	100
3. $MgCl_2$	22	71.4	71.4
4. $CaCl_2$	16	23.3	23.3
5. NaCl	15	15.3	15.3
6. $MgCl_2 + CaCl_2$	32	147.6	76.2
7. $KCl + CaCl_2$	30	131.9	31.2
8. $MgCl_2 + NaCl$	24	90.9	19.6
9. $KCl + NaCl$	17	31.2	...
10. $KCl + MgCl_2$	14	7.6	...
11. $CaCl_2 + NaCl$	8	...	...
12. $KCl + CaCl_2 + NaCl$	45	247.6	116.4
13. $MgCl_2 + CaCl_2 + KCl$	42	223.2	75.6
14. $KCl + MgCl_2 + NaCl$	33	155.5	141.5
15. $MgCl_2 + CaCl_2 + NaCl$	31	134.6	...
16. $KCl + CaCl_2 + NaCl + MgCl_2$	52	300.0	52.4

17.	Combination of No. 16 + $MgSO_4$	58	347.6	47.6
18.	„ „ „ + $(NH_4)_2SO_4$	55	322.3	22.3
19.	„ „ „ + $FeSO_4$	53	307.6	7.6
20.	KCl + $CaCl_2$ + $MgSO_4$	55	323.2	195.3
21.	Combination of No. 16 + $MgSO_4$ + tartaric acid.	65	400.0	52.4
22.	Combination of No. 16 + $MgSO_4$ + citric acid	63	382.0	35.7
23.	Combination of No. 16 + $MgSO_4$ + ammonium tartrate	110	747.6	400.0
24.	Combination of No. 16 + $MgSO_4$ + ammonium citrate	104	706.0	358.4
25.	KCl + $CaCl_2$ + $MgSO_4$ + amygdalin	54	315.8	...
26.	„ „ „ + tannin	57	338.4	15.2
27.	„ „ „ + methylamine	72	452.6	129.4
28.	„ „ „ + quinine	60	362.5	39.3
29.	„ „ „ + strychnine	58	347.6	24.4
30.	„ „ „ + aspartic acid	87	571.4	248.4
31.	„ „ „ + glutamic acid	76	490.0	167.7
32.	„ „ „ + asparagin	142	992.5	669.3
33.	„ „ „ + glutamine	125	862.5	539.3
34.	„ „ „ + peptone	180	1290.9	967.7

Series 12. Comparative Experiments with Natural and Artificial Media.

In order to compare the results obtained with artificial media to that of natural media, such as grape juice and malt-wort, a number of different combinations have been tried. Accordingly, cultures have been made with grape juice of 10.11° and 2.5° Beaume′ with or without nutrient salts, so as to show the effects of salts to natural media. The results of the experi-

ments are shown below, in which only the third microscopic counting of the yeast cells has been recorded.

Combination.	Number of yeast cells in million.
Synthetic solution	9
„ „ +salts of series 7 (.016 M KCl+ .01 M $CaCl_2$+.005 M $MgSO_4$	52
„ „ +Peptone	125
„ „ + „ + salts of series 7	180
„ „ + salts of series 8 (.016 M KCl+ .01 M $CaCl_2$ +.001 M NaCl+.005M $MgCl_2$+ .005M $MgSO_4$+.01M $(NH_4)_2C_4H_4O_6$)	110
Malt-wort	122
Grape juice (10.11°Beaume)	195
„ „ „ +salts of series 7	140
„ „ „ +peptone	315
Dilute grape juice (2.5°Beaume)	145
„ „ „ +salts of series 7.	185
„ „ „ +peptone	225
„ „ „ + „ + salts of series 7.	300

From the above table it is evident that .5 gram peptone with synthetic solution alone allowed a growth of 125 millions which is almost equal to malt-wort and approaches that of dilute grape juice, being 122 and 145 millions respectively. Besides, the combination of .5 gram peptone with the salts of series 7 (.016M KCl+.01 M $CaCl_2$+.005 M $MgSO_4$) in synthetic solution is almost equal to that of grape juice, being 180 and 195 millions respectively. This clearly shows that the artificial medium with peptone is almost equal to the natural medium in regard to fermentation and multiplication of yeast cells.

Furthermore, a combination of dilute grape juice with .5 gram pedtone allowed a growth better to a certain extent than grape

juice alone, being about 225 and 195 millions respectively; but when the salts of series 7 are added to dilute grape juice with peptone the medium exhibits a still better growth of about 300 millions.

It is also evident from the above table that the addition of salts of series 7 to grape juice lowered the growth of the organism to 140 millions, while grape juice alone gave a growth of 195 millions. But the addition of peptone to grape juice stimulated the growth to 315 millions. On the other hand, dilute grape juice allowed a growth of 145 millions, but when the salts of series 7 were added the growth was enhanced to 185 millions. Moreover, the addition of peptone to the above combination stimulated the growth to 300 millions. This clearly proves that the addition of inorganic salts to a physiologically balanced nutrient solution, such as grape juice, is detrimental to the growth of yeast, while the same salts in a dilute medium stimulates the growth of the organism.

It may also be pointed out here that the physiologically balanced solution, as has been shown in series 8 (ammonium tartrate), allowed a growth up to 110 millions, which is comparable to a certain extent to that of peptone alone with synthetic solution as well as that of malt-wort and dilute grape juice. being 125, 122 and 146 millions respectively.

Relative Osmotic Pressure of salts used.

In a quantitative study of toxic ane antagonistic effects of salts on an organism in artificial nutrient media, it is always necessary to use chemically pure salts, either dry or containing a definite amount of water. In all the experiments conducted on yeast Baker's analysed salts were used. It must be admitted here that magnesium sulphate, being very deliquescent, absorbs so much mositure as to make it almost impracticable to weight accurately. In order to avoid this error it was dried at 100° to 105° C in a

dry oven for an hour every time after use and the cork of the bottle was kept air tight by melted paraffin.

It has been shown by investigators, especially Livingston, Tottingham and Shive, that a culture medium may influence plant growth by its osmotic concentration. The osmotic pressure of synthetic solution with added salts, used in yeast culture, produces effects similar to those of other plants, but of less degree. It is necessary therefore to know the osmotic pressure of solutions used in any physiological experimentation.

Experiments have shown that the chemical properties of a solution vary a great deal according to the uature and proportions of the solutes present in the solvent. It has been definitely shown by Tottingham[47] and shive [122] that the osmotic properties in certain proportions in so far as these properties are known to effect plants, are dependent upon the total concentrations and upon the extent to which the component solutes are ionised in the solution, So in order to study the influence of different salts on nutrient media for normal growth of plants, it is necessary to determine the osmotic concentration which to a large exent controls the life of the organism.

In all the experiments to determine the toxic and antagonistic effects of salts on yeast, either singly or in combinations, the purpose of the writer was not to conrol the osmotic concentration of the culture media, but to determine the optimum concentration at which the yeast plants thrives best and also at the same time to find out the effects of some extreme concentrations of different salts that the organissm stands. The osmotic concentrations of single and binary combinations are shown in curves of Figs. 12 and 13 respectively. The osmotic preasure of single salts has been determined from .001 M to .8 M in the case of NaCl and $CaCl_2$, and from .001 M to 1. 7 M & 2.2 M in the case of $MgCl_2$ and KCl respectively. In the binary combinations the first salts in each case has been taken from .001 M to .4 M,

while the second has been used at a definite concentration of .01 M.

The osmotic pressure of each salt both singly and in combinations has been approximately determined by a Beckmann thermometer. The lowering of the freezing point was determined for all the different series, and the osmotic pressure was calculated from the depression of temperature in each case according to the formula given *Jones. It may be added here that there is a great deal of difference between different salts at the same concentration either singly or in combination.

The osmotic concentration of a solution does not increase at the same rate as the concentration of the solute. For example, .001 M NaCl in a solvent of distilled water has an osmotic pressure of approximately .039 atmosphere, whilst the same salt in the same solvent at a concentration of .01 M and .1M is approximately 1.145 & 4. 279 atmospheres respectively. Besides, thedegree of dissociation, as influenced by different combinations of salts in solution, varies a great deal of which very little is known.

The curves in Figs. 14 and 15 show the approximate osmotic pressure of a few of the ternary and quaternary combinations of salts used in the experiments with yeast. In the case of ternary salts the first two salts have been taken at a constant ratio of (1) .016 M KCl + .01 M $CaCl_2$, (2) .01 M KCl + .015 M $MgCl_2$, and (3) and (4) .022 $MgCl_2$ + .01 M $CaCl_2$, while the third salt has been added from .001 M to .4 M in each case, as is shown in Fig. 14. Similarly, in the case of quaternary salts the first three salts have been added at the constant ratio of .016 M CaCl + .01 M KCl_2 + .001 M NaCl, while the fourth has been taken from .001 M to .4 M , as is shown in Fig. 15.

*Jones, H. C., Elements of Physical Chemistry. P. 256.

Fig 12.

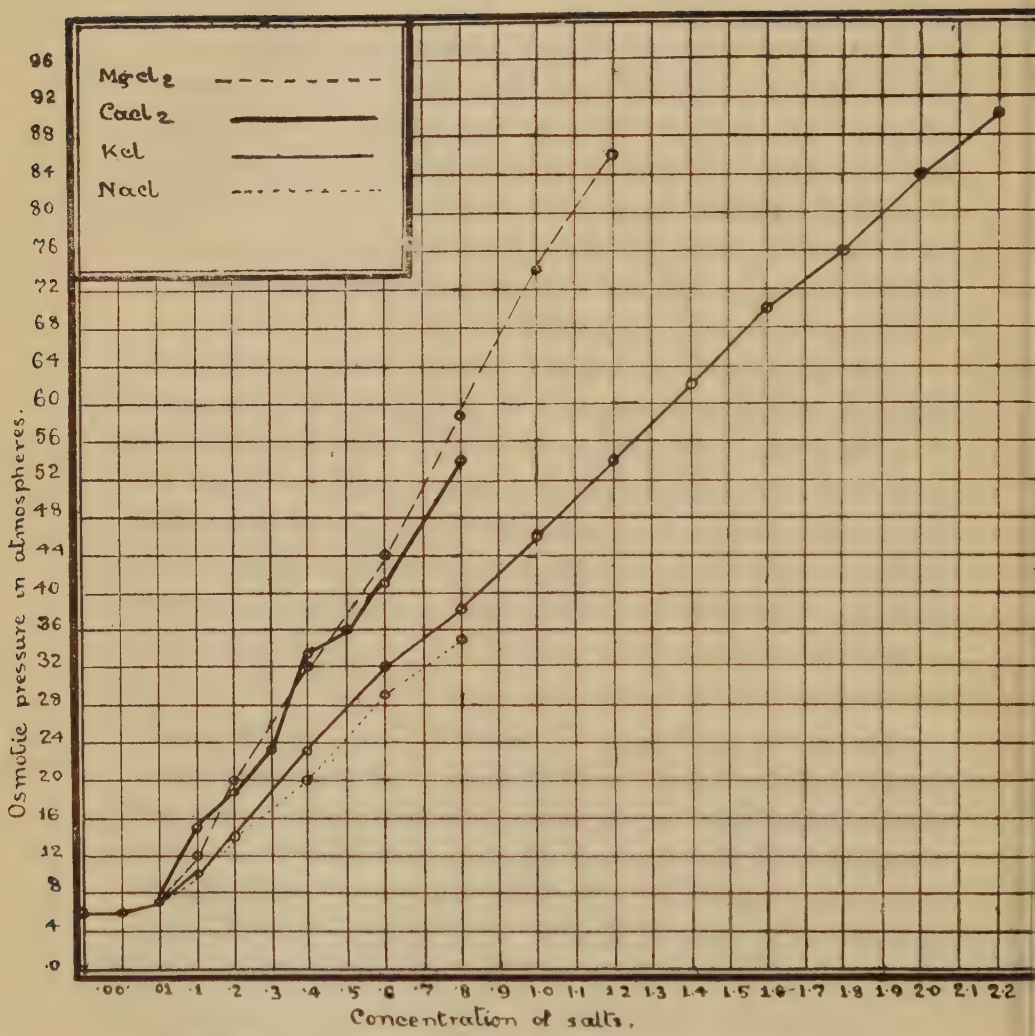

FIG. 12. Curves showing the osmotic pressure of KCl, MgCl, $CaCl_2$ and NaCl with synthetic solotion as solvent. The ordinates represent the osmotic pressure in atmospheres, ond the apscissae, the consentration of salts. The ordinate :at 6 represents the osmotic pressure of the synththetic solution alone.

Although it is the total concentration of salts consisting of both electrolytes and non-electrolytes and not its component parts alone that controls the growth of the organism in a culture medium yet the total osmotic concentration of the electrolytes, *i.e.*, the nutrient salts, is a more important factor in yeast culture than that of non-electrolytes, which in this case are the fermentable sugars in the synthetic medium.

Fig 13.

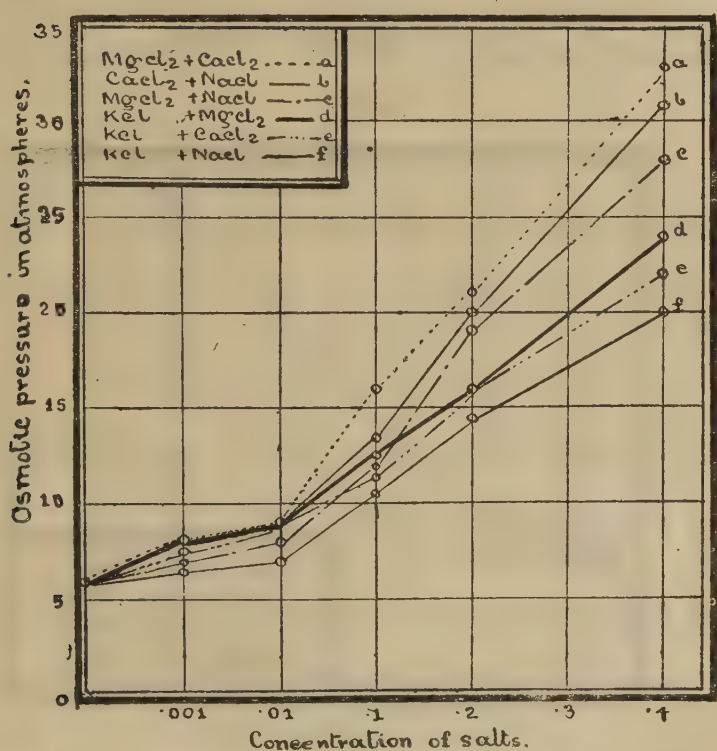

Fig. 13. Curves showing the osmotic pressure of binary combinations of KCl, $CaCl_2$, NaCl and $MgCl_2$ with synthetic solution as solvent. The ordinates represent the osmotic pressure in atmospheres and the adscissae, the concentration of salts in combination. The ordinates at 6 represents the osmotic pressure of synthetic solution alone.

The curves in Fig. 16 show the relative osmotic concentration of different salts that have been used in developing a physiologically balanced nutrient solution for yeast. The combinations used are as follows:—

(1) .016 M KCl.+.01 M $CaCl_2$ + .001 M NaCl+.005 M $MgCl_2$ +.001 M $MgSO_4$ to .1 M $MgSO_4$.

(2) .016 M KCl+.01 M $CaCl_2$ +.001 M Mg SO_4. to M Mg SO_4.

(3) .016 M KCl+.01 M $CaCl_2$ +.001 M NaCl + .005 M $MgCl_2$ + .005 M $MgSO_4$ + .001 M to .1 M $(NH_4)_2C_4H_4O_6$

Fig 14.

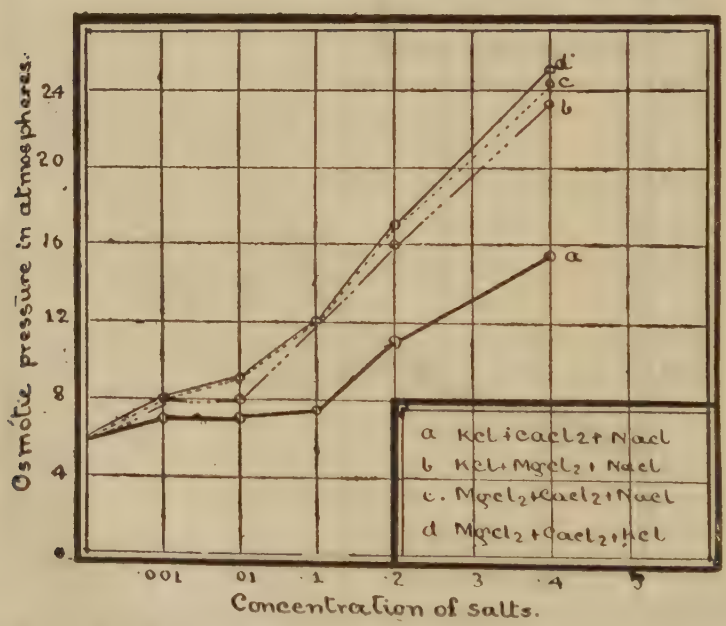

Fig. 14. Curves showing the osmotic pressure of ternary combinations of KCl, $CaCl_2$, NaCl and $MgCl_2$ with synthetic solution as solvent. The ordinates represent the osmotic pressure in atmospheres and the abscissae, the concentration of salts in combination. The ordinate at 6 represents the osmotic pressure of synthetic solution alone.

(ammonium tartrate). (The last salt in each case has been added from .001 M to .1 M).

The total osmotic pressure of the medium that gave the best growth with yeast in series 8 (ammoninm tartrate) is approximately 7.795 atmospheres, of which the osmotic pressure of sugar (5% sugar solution) alone is 3.631 atmospheres. Therefore the osmotic pressure of total salts with phosphoric acid and

Fig 15.

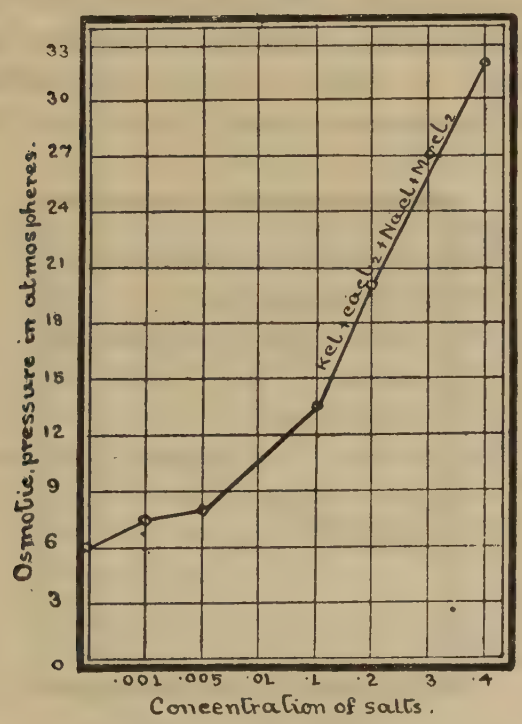

Fig. 15. A curve showing the osmotic pressure of the quaternary combinations of KCl, $CaCl_2$, NaCl and $MgCl_2$ with synthetic solution as solvent. The ordinates represent the osmotic pressure in atmospheres and the abscissae, the concentration of salts in combination. The ordinates at 6 represents the osmotic pressure of synthetic solution alone.

ammonium hydroxide used in synthetic solution is approximately 4.164 atmospheres. Even an increase in percentage of sugar in the synthetic medium up to 10% (5.55°Beaume) does not materially change the rate of growth in presence of the same concentration of salts, but, if the osmotic pressure of the medium is raised by the addition of nutrient salts the growth is arrested very markedly.

It is with a view to determine the effects of osmotic pressure of both the nutrient salts and the fermentable sugars, *i.e.*, electrolytes and non-electrolytes, on yeast that a number of flasks were arranged having the synthetic solution as media with the nutrient salts of series 8 (ammonium tartrate). In one series the osmotic

Fig 16.

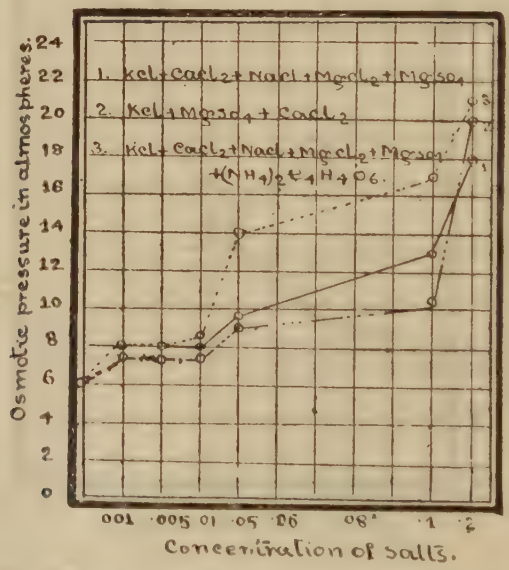

Fig. 16. Curves showing the osmotic pressure of three different combinations of salts with the synthetic solution as solvent. The ordinates represent the osmotic pressure in atmospheres, while the abscissae, the concentration of salts in combination. The ordinate at 6 represents the osmotic pressure of the synthetic solution alone.

pressure was varied approximately from 8 to 80 atmospheres by the addition of cane sugar (5% to 60%) while the concentration of nutrient salts remained the same as in the synthetic solution with series 8. In another series the flasks were arranged in the same way, except that the osmotic pressure of the synthetic medium was raised by proportionally increased concentration of nutrient salts approximately from 8 to 50 atmospheres, while the concentration of sugar remained the same as in the synthetic medium with series 8. In both cases the osmotic pressure of the synthetic medium with the nutrient salts of series 8 (ammonium tartrate), which was used as a check, was approximately 8 atmospheres. The results of the experiments are shown in curves of Fig. 17, which are the average results of duplicate flasks.

From the curves it is evident that the highest growth obtained in this case is at the osmotic pressure of 15 and 8 atmospheres in the case of sugar and salt respectively. The decline in rate of growth in the latter case is very marked. The yeast cells are found to bud even at the osmotic pressures of approximately 60 and 30 atmospheres in the case of sugar and salt respectively, beyond which no appreciable growth was noticed. However, in such a high osmotic pressure the fermentation was entirely checked.

It may be stated here that grape juice which contains 20% total solid and whose osmotic pressure is approximately 33 atmospheres, allowed the maximum growth of yeast. From this it is evident that the controlling factor in the growth of yeast in these experiments is not the osmotic pressure, but the chemical effects of the nutrient salts used.

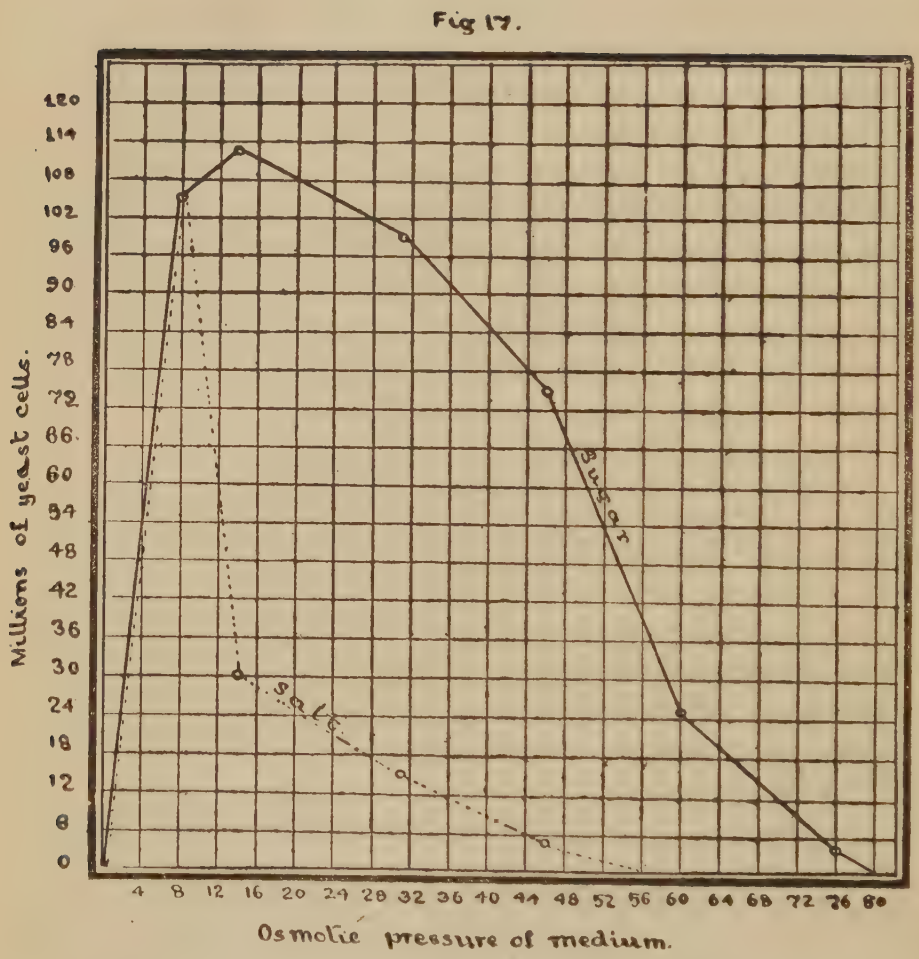

Fig. 17. Curves of yeast growth showing the effects of osmotic pressure of the synthetic medium produced by the addition of sugar and salts respectively. The ordinates represent the number of yeast cells in millions and the abscissae, the approximate osmotic pressure in atmospheres of the synthetic medium.

Summary.

1. As shown by the multiplication of yeast cells the variations in antagonism of ternary and quaternary combinations of the four metallic chlorides may be arranged as follows:—

Ternary Combinations.

1. $KCl + CaCl_2 + NaCl$ (most)
2. $MgCl_2 + CaCl_2 + KCl$
3. $KCl + MgCl_2 + NaCl$
4. $MgCl_2 + CaCl_2 + NaCl$ (least)

Quaternary Combinations.

1. $KCl + CaCl_2 + NaCl + MgCl_2$ (most)
2. $KCl + MgCl_2 + NaCl + CaCl_2$
3. $NaCl + MgCl_2 + CaCl_2 + KCl$
4. $CaCl_2 + MgCl_2 + KCl + NaCl$ (least)

The multiplication of yeast cells in the various quaternary combinations was almost identical.

2. The effects of ternary and quaternary combinations of salts on yeast, whether positively or negatively antagonistic in comparison to other plants, bacteria and animals, may be tabulated as follows:—

Ternary salts.	Yeast.	Other plants.	Bacteria	Animals.
No. 1	—|—	—|—	.....	—|— and—
„ 2	—|—	+	.....	——
„ 3	+	+ and ——	—|—	+ and——
„ 4	—— and +	+ and ——	—|—	+ and——
Quaternary Salts.				
No. 1 to 4.	—|—	—|—	—|—*	—|— and —

* —\|— Strong antagonism
\+ Mild antagonism
—— Strong increase of toxicity.
– Slight increase of toxicity.

3. Among the three sulphates used magnesium sulphate is better suited to yeast in synthetic media than ammonium sulphate. Iron sulphate has the least effect on the growth of the organism.

4. The combinations of .016 M KCl+.01 M $CaCl_2$+.001 M $NaCl$+.005 M $MgCl_2$+.005 M $MgSO_4$ and of .016 M KCl+.01 M $CaCl_2$+.005 M $MgSO_4$ are both physiologically balanced salts with the synthetic solution for yeast, of which the former is better suited than the latter.

5. Of the two organic acids tartaric acid is more preferred by yeast than citric acid.

6. Ammonium tartrate has a decided stimulating influence on yeast. The combination of .016 M KCl+.01 M $CaCl_2$+.001 M $NaCl$+.005 M $MgCl_2$+.005 M $MgSO_4$+.01 M $(NH_4)_2C_4H_4O_6$ (ammonium tartrate) with synthetlc medium has been found to be a physiologically balanced nutrient solution for yeast. Besides the combination of .016 M KCl+.01 M $CaCl_2$+.005 M $MgSO_4$+.01 M $(NH_4)_2C_4H_4O_6$ (ammonium tartrate) is also a physiologically balanced nutrient solution for yeast. Ammonium citrate behaves in a similar way as amonium tartrate, though not to the same extent.

7. The deterioration of yeast cells, after fermentation is over, is mainly due to the increase of acidity in the medium during the fermentation.

8. The organic substances, such as amygdalin, tannin, quinine and strychnine, have little influence on yeast. They are more or less toxic in their behaviour on the organism.

9. The amines and the amino acids have stimulating influence on yeast. Their behaviour to the organism is almost the same, although amino acids are better suited for growth than the amines. Of the two amino acids, aspartic acid is more favourable than glutamic acid.

10. Both asparagin and glutamin increase the growth of yeast, the former more than the latter.

11. Peptone greatly increases the growth of yeast. Its influence is more pronounced than any other nitrogenous substance used. The combination of peptone plus the nutrient salts with the synthetic medium causes a growth almost equal to that of grape juice.

12. In relation to the osmotic pressure of the medium the yeast plant does not respond like other plants. It is the osmotic pressure of electrolytes (added salts) rather than the non-electrolytes (fermentable sugar) that controls the growth of the organism in synthetic media.

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AUTOBIOGRAPHY.

I, Swarna Kumar Mitra, was born in Dacca, Bengal (India) February 15, 1887. I received practically Secondary school education in the High English School of Abdullapur, Dacca (Calcutta University) ; my undergraduate education at the University of california from which I obtained the degree of Bachelor of Science in 1915 and the Degree of Master of Science in 1916. In 1920 I received from the Ohio State University, the Degree of Doctor of Philosophy after a two years' residence on a University Fellowship.

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